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Background to Chemotherapy

HISTORY OF CHEMOTHERAPY
THERAPEUTIC APPLICATION OF MICROBIAL ANTAGONISMS
ANALYSIS OF ANTIBACTERIAL ACTION
NUTRITION OF BACTERIA
MICROBIAL RESISTANCE TO CHEMOTHERAPEUTIC DRUGS
CHEMOTHERAPY OF RICKETTSIAL AND VIRUS DISEASES
PRINCIPLES AND PRACTICE OF LOCAL CHEMOTHERAPY
PRINCIPLES OF ADMINISTRATION IN CHEMOTHERAPY

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CHEMOTHERAPY AND THE INSTITUT PASTEUR
CLAUDE BERNARD

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Background to Chemotherapy

CHEMOTHERAPY has reached a stage at which there is a tendency for general principles to be obscured by a multiplicity of details. This number of the Bulletin is entitled " Background to Chemotherapy " because it is concerned not with the practical applications of chemotherapy, but with some aspects of its historical development and theory. With such an approach to the subject, one of the first questions must be : what is chemotherapy ? To this question it is possible to find almost as many answers as there are authorities. Some would exclude, while others include, from the definition the local application of drugs otherwise described as chemotherapeutic. Some would limit the term to the treatment of infections, while others would include the experimental treatment of cancer by chemical compounds. Some would agree with a contributor to this number of the Bulletin, who says that " the use of antitoxins constitutes an important part of chemotherapy ", while others would dissent vehemently from this view. Only one conclusion is possible from these conflicting definitions : that the word chemotherapy, as it is currently used, has no precise meaning.

Whatever chemotherapy may be, Ehrlich is by common consent its father, and it is perhaps of interest to consider the sense in which he used the term. In a lecture to the Berliner Medizinische Gesellschaft on 13 February 1907, he said : " What we want is a *Chemotherapia specifica*, that is, we are looking for chemical agents which, on the one hand, are taken up by certain parasites and are able to kill them and, on the other hand, in the quantities necessary for this lethal action, are tolerated by the organism without too great damage." In the same lecture, Ehrlich expanded this notion by referring to the properties of drugs as bacteriotropic—or, more generally, aetiotropic—and organotropic. Drugs were properly described as specific only if their aetiotropy was stronger than their organotropy.

The essence of Ehrlich's conception of chemotherapy was specificity. The conception originally owed nothing to the treatment of infections, but arose from attempts to exploit the affinity of methylene blue for nervous tissue in the treatment of neuralgia. In a paper on " the analgesic action of methylene blue ", Ehrlich wrote in 1890 of " cellular therapy " as " a consequence of specific cell-nutrition ", and added that " only those substances can really influence the cell that are, if the expression may be permitted, eaten by it ".

In another paper of 1907 on " Biological therapy ", Ehrlich speaks of a *Therapia distributiva*, and in 1913, in his " General address of chemio-therapy " at the XVIIth International Congress of Medicine, he lays down as the principle of chemotherapy : *Corpora non agunt nisi fixata.*

Although he had earlier used the term *specific chemotherapy*, Ehrlich came to use the more general *chemotherapy* to express a complex of ideas, which included : first, effective *distribution* to the seat of action ; second, *fixation* of the drug by the cell or tissue, due to a particular affinity between them ; third, and possible only after the fulfilment of the first two conditions, the *specific action* of some part of the drug on the cell, which was a property distinct from the affinity which resulted in fixation. Because Ehrlich's brilliant conceptions found their most fruitful expression in the treatment of microbial disease, the term *chemotherapy* has come to stand almost exclusively for *specific antimicrobial chemotherapy*, or, more broadly, according to a recent definition (McIlwain, *Biol. Rev.* 1944, **19,** 135), for " a subject concerning the properties and the various interactions of drug, parasite, and host ".

The simpler idea of chemical sterilization of the infected organism preceded that of specific antimicrobial chemotherapy. Thus, C. Macnamara (*Practitioner*, 1871, **6,** 72), only four years after Lister's introduction of surgical antisepsis, employed phenol systemically on the ground that it " upon anti-dry-rot principles may protect the body from suppuration ". Later, Koch treated animals infected with anthrax by injecting mercuric chloride, but succeeded only in killing the hosts.

From these considerations, we are led to the conclusion that the unqualified term chemotherapy is inadequate. In any branch of science, it is axiomatic that a term should be used by different persons as a symbol for the same thing or idea. Thought and word are inextricably associated, and a looseness or inconsistency of terminology necessarily implies some underlying imprecision of ideas. But whatever may be its most suitable designation, the extent and depth of the subject (or subjects) usually regarded as pertaining to " chemotherapy " are indicated by the articles which follow.

* * *

DR. E. M. LOURIE, who contributes an introductory article on the historical development of chemotherapy, is director of the Warrington Yorke Department of Chemotherapy at the Liverpool School of Tropical Medicine. He was formerly associated with Professor S. Adler in work on sandflies and leishmaniasis in Palestine, Iraq and Persia, and was then appointed assistant lecturer in protozoology at the Liverpool School of Tropical Medicine. He was a Rockefeller Research Fellow in the University of Chicago from 1931-1933, where he worked on the chemotherapy of malaria in the Department of Professor W. H. Taliaferro. He later worked again at the Liverpool School of Tropical Medicine, first as a Beit Memorial Research Fellow and then as a member of the scientific staff of the Medical Research Council. During this time he was associated with the late Professor Warrington Yorke in the development of the diamidines as chemotherapeutic agents against protozoal infections [see article by Warrington Yorke on this subject (*BMB* **293**)] and for a few years he was seconded to the Colonial Medical Service, in Sierra Leone, West Africa, in connexion with a local outbreak of sleeping sickness. On the creation of a department of chemotherapy, as a memorial to Warrington Yorke, at the Liverpool School of Tropical Medicine in 1943, he was appointed its first director.

SIR HOWARD W. FLOREY received a knighthood in 1944, and was awarded the Nobel Prize for Medicine, with Dr. E. Chain and Sir Alexander Fleming, in 1945. A short note on his earlier work appeared in a previous number (vol. 2, No. 1) of the Bulletin, and his work on antibiotics is too familiar to require mention here. Sir Howard Florey is, at the time of going to press, visiting South America under the auspices of the British Council.

DR. HENRY MCILWAIN is a member of the Scientific Staff of the Medical Research Council, and is at present working in the Council's Unit for Research in Cell Metabolism at Sheffield University ; he is also lecturer in biochemistry in the University. His research has been mainly on chemical and biochemical aspects of microbiological problems, commencing in Newcastle and Oxford with the isolation and synthesis of bacterial products. This was followed by investigations of bacterial nutrition, with the Medical Research Council's Department of Bacterial Chemistry (the work of which is described by Sir Paul Fildes, its director, in these columns) at the Middlesex Hospital, London ; and by studies of biochemical processes associated with microbial growth, while in Sheffield. Many of the latter investigations have been connected with chemotherapeutic problems and have illustrated a method of designing antibacterial agents, and included studies of the modes of action of several chemotherapeuticals. References to these will be found in *Nature*, 1943, **151,** 270 ; 1944, **153,** 300 ; and in *Biological Reviews*, 1944, **19,** 135, and subsequent papers in the *Biochemical Journal*. Dr. McIlwain is a Fellow of the Chemical Society, and a member of the Biochemical Society and of the Society for General Microbiology.

SIR PAUL FILDES was for many years assistant bacteriologist to the London Hospital under the late Professor William Bulloch. With Bulloch he was the author of the standard work on haemophilia in Karl Pearson's *Treasury of human inheritance* (1911). He was associated with Professor James McIntosh in pioneering the Wassermann reaction and " 606 " in Britain, and formulated a new outlook on " parasyphilis " with McIntosh and the late Sir Henry Head. On the outbreak of the first World War he and McIntosh turned to anaerobes and produced the McIntosh-Fildes jar. He served in the Navy (Surgeon Lt.-Comdr., R.N.V.R.) in charge of the Pathological Laboratory at the R.N. Hospital, Haslar, where he continued to study syphilis, cerebrospinal fever and influenza. In the latter connexion, he designed " Fildes' agar " for the growth of the bacillus, and was one of the first to throw light on the " growth factors " of *B. influenzae*. Returning to the London Hospital, he took up the study of tetanus and originated the idea that germination of spores depended upon a suitably low oxidation-reduction potential in the cultures or tissues. This led to the formation of the Medical Research Council's Unit for Bacterial Chemistry. Fildes was one of the authors of the Medical Research Council's volume, *Diphtheria*, general editor of its *System of bacteriology*, and one of the founders of the *British Journal of Experimental Pathology*, of which he has been editor or director from the beginning. He is a member of the usual pathological societies and a Fellow of the Royal Society.

DR. F. R. SELBIE was educated at Aberdeen University, and after spending a year as House Physician to the late Sir Ashley W. Mackintosh, he was awarded the Alexander Anderson Scholarship of Aberdeen University, with which he proceeded to the Pasteur Institute, Paris, to work in the laboratories of Professor C. Levaditi from 1929 to 1931. He then became Scientific Assistant to the Director of the Imperial Cancer Research Fund, London, until he was appointed to the staff of the Bland-Sutton Institute of Pathology, Middlesex Hospital, London, in 1936, where he has been assistant pathologist for 7 years. His work with Professor Levaditi was mainly concerned with the pathology and chemotherapy of spirochaetal injections and the properties and pathogenicity of *Streptobacillus moniliformis*. In his later work he has been engaged mainly on chemotherapy, virus studies and cancer research, both independently and in collaboration with Professor James McIntosh, who, with Sir Paul Fildes, was the first to introduce salvarsan therapy to Britain. He has recently been working on the development of resistance and other factors which interfere with the action of drugs on bacteria ; the treatment of experimental and clinical infections with penicillin, the sulphonamides, the acridines and other drugs ; and the phenomena of interference and superinfection in animal virus diseases.

DR. C. H. ANDREWES is one of the world's principal authorities on virus disease in animals. A note on his work appeared in an earlier number (vol. 2, No. 12) of the Bulletin.

DR. HAROLD KING is an authority on medicinal substances. He was educated at Bangor University and from 1912 to 1919 worked in the Wellcome Physiological and Chemical Laboratories. From 1919 he has been on the Staff of the Medical Research Council at the National Institute for Medical Research. King has carried out notable work in the field of alkaloids, in particular on hyoscine and the curare group of alkaloids, and is well known as an investigator on the chemotherapy of trypanosomiasis and of malaria. With Rosenheim he put forward the *cyclo*pentanophenanthrene formula which has revolutionized the chemistry of the sterols, sex-hormones, heart-poisons and related substances.

DR. L. P. GARROD is bacteriologist to St. Bartholomew's Hospital and professor of bacteriology in the University of London. He is particularly well-known for his work on antibacterial drugs, a subject to which he had devoted considerable attention before the introduction of sulphonamides. He has previously been the subject of a note (vol. 1, No. 3), in this Bulletin, to which he has contributed on two previous occasions (*BMB* **48, 197**).

DR. J. H. GADDUM worked under J. W. Trevan and H. H. [now Sir Henry] Dale before he became professor of pharmacology in Cairo. He was then appointed to the chair of pharmacology at the Pharmaceutical Society, London, and a few years ago became professor of pharmacology at Edinburgh. He has written papers on the application of mathematical methods in biology and on the use of pharmacological methods for the estimation of substances such as histamine, acetylcholine and adrenaline in the tissues and body-fluids. Most of our present knowledge of the physiological importance of these substances is based on pharmacological assays. The pharmacological department at Edinburgh is now active in this field. Professor Gaddum is the author of a textbook of pharmacology which provides for medical students and others a clear guide to the important principles of the subject. The book was immediately recognized as possessing many advantages, and it has in a short time become a standard work.

A SKETCH OF THE HISTORY OF CHEMOTHERAPY

E. M. LOURIE, M.B., D.P.H.

Director of the Warrington Yorke Department of Chemotherapy
Liverpool School of Tropical Medicine

The word "chemotherapy" means, literally, the use of chemical substances for treating the sick. This would cover a vast field in the practice of medicine, including the use of quite simple compounds such as sodium bicarbonate or aspirin for dyspepsia or headaches, and more complex substances such as vitamins or hormones for deficiency-diseases or endocrine disorders. The term should, however, be restricted in its meaning to the intention of its originator, Paul Ehrlich, that is, to the treatment only of *infections* (and perhaps cancer) by chemical compounds. As has been well stated by Schulemann (1939), "the investigator in chemotherapy strives after somewhat different aims [from those of the pharmacologist]. He is not concerned with restoring diseased cells to their normal function or with bringing about a reversible alteration of the cell-functions. He aims at reaching the parasite-cells and at throwing their function so irreversibly out of order that the parasite dies but the host remains as unharmed as possible". Chemotherapy is thus at the same time a department of pharmacology and an extension of that subject. It is a part of pharmacology in so far as it is concerned with the action of drugs on the infected subject; and it lies outside a strict conception of pharmacology in so far as it is concerned with the action of drugs on the parasite. In fact, some of the more striking modern advances in chemotherapy derive from studies of drug-action on the parasite, protozoon or bacterium, in the test-tube, isolated from its host, though the ultimate picture can be obtained only by complementary studies both *in vitro* and *in vivo* (McIlwain, 1943a).

Origins of Chemotherapy

Texts on the history of medicine have acclaimed as the Father of Chemotherapy that colourful, robust, hard-drinking, quarrelsome rebel against authority, whose name, in keeping with his flamboyant character, was Aureolus Theophrastus Bombastus von Hohenheim (1493-1541). Generally known as Paracelsus, one account has it that he himself chose this name, with characteristic self-esteem, in order to signalize his parity with Celsus, one of the great physicians of antiquity. The progress of medicine had been delayed for thirteen hundred years by unquestioning servitude to the teachings of Galen, and it was the outstanding service of Paracelsus that he finally broke the bonds of this powerful tradition. He startled and outraged the academic world by inaugurating his appointment as professor of medicine in Basle by publicly burning the works of Galen, and by lecturing in German for all to understand, instead of in Latin for the favoured few. While discarding the doctrine preached by Hippocrates of the four humours—blood, phlegm, black bile and white bile—he reintroduced that great teacher's method of practising as far as possible in the light of experience and exact observation rather than from the books of traditional authority.

It is perhaps largely because of his vigorous championing of antimony, and other elements, for all manner of ills, and because of his reputed introduction of mercurials for the treatment of syphilis (Garrison, 1929), that claim may be made for Paracelsus as the founder of chemotherapy. However, although he infused some measure of honesty and chemistry, in place of humbug and alchemy, into the practice of medicine, his outlook and methods were inevitably very heavily tainted with the magical and irrational ideas of his age. Indeed, medicine seems, no doubt because of its subjective quality, always to have lagged well behind other sciences in discarding magical precepts. It is significant that the rational approach of Robert Boyle (1627-1691), epitomized in *The sceptical chymist*, earned him recognition as one of the originators of the scientific method; yet, when writing on therapeutics, he could warmly recommend mixtures containing worms, horse dung, human urine, and moss from a dead man's skull (Clark, 1940).

Let us then place Paracelsus as no more than the somewhat cautiously acknowledged grandfather of chemotherapy, whilst having no doubts about referring to Paul Ehrlich (1854-1915) as the father of modern and scientific chemotherapy. With the clear foundations of microbial parasitism introduced by Pasteur (1822-1895), and with his own studies on the chemistry and selective vital staining properties of dyes to build upon, Ehrlich constructed an intricate and ingenious edifice of theory, together with considerable practical achievement. By imagining parasites to be endowed with particular points of attachment, chemo-receptors, with a special affinity for specific drugs, and by embellishing this concept with all manner of improvisations to meet the vagaries of his experimental results, he created a fertile and provocative field for experiment and argument, highly stimulating to subsequent workers in chemotherapy up to the present day. Ehrlich's greatest practical achievement was the introduction of salvarsan (now known as arsphenamine) for syphilis in 1910. This was, however, not the only infection for which a specific remedy was by this time known. Cinchona bark and quinine had already long been recognized as remedies for malaria, and ipecacuanha and emetine had also already been established as specifics for amoebic dysentery. In the field of veterinary medicine, arsenical compounds were already in use for trypanosome infections, and Nuttall & Hadwen (1909) had introduced trypan blue for babesia infections.

The chemotherapy of spirochaetal and protozoal diseases now made great strides, but it was not until the introduction of the sulphonamides by Domagk in 1935 that bacterial infections came well within the range of chemotherapy. This was a turning point in our history, since all previous efforts to devise chemotherapeutic agents against bacterial infections had been so consistently disappointing that the belief was growing that such infections were inherently insusceptible to treatment by chemotherapy. The next and latest high peak of achievement was the discovery of penicillin by Fleming (1929) and of its therapeutic application by Florey (Chain, Florey, Gardner, Heatley, Jennings, Orr-Ewing & Sanders, 1940). This was the culminating triumph in a long history of the investigation of the by-products of micro-organisms as possible therapeutic agents. Elsewhere in these columns Florey (1946) traces this history as far back as sixty or seventy years ago, when a number of workers, including

Pasteur & Joubert (1877), observing that one micro-organism can elaborate a substance harmful to another of different species, remarked on the potential significance of this for therapeutics. Indeed, there are several contemporary accounts of the successful protection of animals from anthrax bacilli by the inoculation of bacteria antagonistic to these pathogens.

Some Omissions from this Survey

A survey as brief as this must necessarily be highly selective. The first sections below will deal with protozoal and spirochaetal infections, which practically monopolized the field of chemotherapy until the introduction of the sulphonamides, but no account will be given of the chemotherapy of leishmaniasis, in which there have been some spectacular successes, and no further mention will be made of amoebiasis. There will also be no further reference (except in connection with syphilis) to the history of penicillin and other antibiotics, since this is fully covered elsewhere in the present issue of this journal (Florey, 1946). The chemotherapy of helminthic infections, of wounds, and of cancer will also not be mentioned, and, needless to say, in each section below there will inevitably be obvious omissions of much that is important.

The Chemotherapy of Malaria

It seems that we must now abandon the oft-told tale of the introduction of cinchona-bark into Europe by the wife of the Viceroy of Peru, the Countess Anna de Chinchon, after she had been cured of malaria in Lima about three hundred years ago. It now appears that this lady died in Spain before her consort went to South America. The Viceroy's second wife, who was with him in Lima, never suffered from malaria ; furthermore, she died on her way home, and therefore cannot be credited with having brought back the bark herself (Haggis, 1941).

Certain it is, however, that Jesuit missionaries and priests played a leading role, from about this period, in the importation of the bark into Europe. In spite of the fact that it was the only true specific known against any disease, its virtues were not universally accepted, and for a very long time there was much bitter opposition to its use. This was probably due partly to the fact that other preparations besides those of cinchona bark were, intentionally or otherwise, frequently confused under the name of Jesuit's bark by the innumerable middlemen engaged in its distribution, and partly to the fact that then, as now, febrile disorders not due to malaria were often wrongly labelled by that name. It was not until the first half of the 19th century that cinchona was fairly generally accepted as a specific for malarial fevers, and quinine and cinchonine, isolated by Pelletier and Caventou in 1820, and the other alkaloids later, were duly recognized as the active agents. About this time nations with Eastern dependencies, in particular the British and the Dutch, became alarmed by the possibility that Cinchona species, indigenous only to South America, might eventually become extinct, owing to the extensive felling of forest trees to meet the rapidly expanding demand. There followed, accordingly, a period of intense endeavour by English and Dutch scientists, explorers and adventurers to transfer seeds or plants to India, Java and other countries. These attempts contained all the ingredients of story-book high adventure, including all manner of rivalries, misunderstandings, disguises, frustration, and violence. Details would take far too long to tell, and suffice it to say that success seemed at first to favour the British, rather than the Dutch. By 1880, the European markets were receiving nearly 20 times more bark from India and Ceylon than from Java (Markham, 1880).

However, the species which grew so well on the Indian plantations, the hardy *Cinchona succirubra*, yields a relatively poor supply of quinine, in relation to total alkaloids, and since the popular demand was specifically for quinine rather than the other alkaloids, these plantations never became an economic success. The eventual ascendancy of the Dutch in this field can be traced to the purchase by their government, for a trifling sum, of a handful of seeds collected in 1865 for an Englishman, Charles Ledger, long resident in Peru. These chanced to be of a type, subsequently named *Cinchona ledgeriana*, which yields an exceptionally high proportion of quinine, but which was very difficult to grow. However, the Dutch succeeded in combining the special virtue of *C. succirubra*, that is its hardihood, with the virtue of *C. ledgeriana*, that is its high quinine-content, by the simple expedient of grafting the one upon the other ; the success of the Java cinchona plantations is, in fact, said to be based on the grafting of millions of *C. ledgeriana* scions on understocks of *C. succirubra* (Taylor, 1945). By the outbreak of the first world war the Java plantations were supplying about 95% of the world's quinine.

In time of war the importance of adequate means of treating malaria is paramount. In 1916, for example, General Sarrail had to report, at a critical period, that his army in the Balkans was immobilized in hospital on account of malaria (Sergent & Sergent, 1932). This was representative of the situation in many theatres in malarious countries, and it is not too much to say that the supply of quinine was one of the essential features upon which the outcome of the first world war depended. After the war an intensive search for synthetic antimalarials was conducted in Germany. With the aid of a technique devised by Roehl (1926) for testing antimalarial activity in canaries, the first notable success was scored in 1924 by the discovery of plasmochin, now known as pamaquin (Schulemann, Schönhöffer & Wingler, 1932), and this was then followed by the more valuable atebrin, now known as mepacrine (Mauss & Mietzsch, 1933).

On the outbreak of the late war, British manufacturers immediately turned their attention to the large-scale production of mepacrine, which, till that time, was a German monopoly. The success of these efforts was one of the deciding factors in winning the war, since the Japanese occupation of Java, with its cinchona plantations, in 1942, immediately threw the armies of the United Nations into jeopardy because of the probability of eventual exhaustion of quinine-supplies, if the war were to last long enough. There followed an anxious period, for whilst it was known that long-continued " suppressive " treatment by quinine is not only safe but would not impair the efficiency of armies in the field, this had not yet been established for mepacrine. Several outbreaks of illness among troops in the field were, in fact, at the time, suspected to be due to the toxic effects of " suppressive " mepacrine treatment, and it became urgently necessary to define exactly the potentialities and dangers of this compound. Its safety and value as a suppressive against all forms of malaria under active service conditions were in due course triumphantly vindicated, and Hamilton Fairley (1945) has described how, among the Australian Forces in New Guinea, for example, its regulated use contributed to a reduction of malaria incidence from 740 per 1,000 per

year in December 1943 to 26 per 1,000 per year in November 1944, with a death-rate of less than 1 in 3,000.

However, there were weighty reasons for a determined search for alternative antimalarials. It was not safe merely to wait for the value of mepacrine to be proved, and among other disadvantages eventually confirmed was the fact that benign tertian malaria is not cured by mepacrine but merely suppressed, only to recur later. Intensive research was therefore pursued in Britain and America, and the most striking result was that achieved in the discovery of paludrine, as the result of an entirely new approach in the search for antimalarials, by Curd, Davey & Rose (1945). It was not possible to obtain canaries in sufficient numbers for routine tests on all the innumerable compounds synthesized, and a method was devised for using young chicks infected with *Plasmodium gallinaceum*, in the preliminary sorting out of potential antimalarials, with resource to other types of malarial infection in chicks and canaries as required.

The hypothesis of James (1931) was adopted, that the inability to effect causal prophylaxis (or radical cure) in benign tertian malaria in man is due to hitherto undemonstrated exo-erythrocytic stages of the parasite, such as were later demonstrated for *P. gallinaceum* (James & Tate, 1937; Huff & Coulston, 1944). The efforts of Curd, Davey & Rose therefore took the direction urged by James (1937), namely, to find a drug which acts not only against the forms occurring in the erythrocytes—and therefore perhaps as a suppressive, or as an ameliorative agent in man—but which acts also against the exo-erythrocytic stages in the bird-infections, and hence as a possible causal prophylactic or radical curative agent in man.

Most of the very considerable synthetic work of earlier investigators in the search for antimalarials had concentrated on variations of either quinoline (as in quinine and plasmochin) or acridine (as in mepacrine), considerably less attention having been paid to other heterocyclic ring systems. Curd, Davey & Rose, however, chose at the outset to explore the possibilities of the pyrimidine nucleus, for a number of novel reasons, and their researches soon led them to lay considerable stress on the conclusion that one of the determinants of antimalarial activity is a molecular configuration which allows of certain types of tautomeric change. It followed from this that if, in pyrimidine compounds selected as exhibiting a high degree of antimalarial activity, the pyrimidine ring were broken in such a way as to leave undisturbed the tautomeric possibilities, antimalarial activity might be retained. On this reasoning the pyrimidine nucleus was discarded, and biguanide compounds emerged, one of which is paludrine, first tested in man by Adams, Maegraith, King, Townshend, Davey & Havard (1945) and Maegraith, Adams, King, Townshend, Davey & Havard (1945). It is too early for this substance to have established its final place in the treatment of malaria, but it is notably non-toxic and acts on all three of the main forms of human malaria. Like mepacrine (but probably more readily), it can prevent infection from becoming established and is a radical cure in malignant tertian, but apparently not always in benign tertian, malaria.

The Chemotherapy of Syphilis

Ehrlich's original experiments leading to the discovery of anti-syphilitic remedies depended on tests against infections in mice caused not by spirochaetes but by trypanosomes; and his eventual introduction of arsphenamine in 1910, followed by neoarsphenamine in 1912, may actually be traced to the demonstration by Wolferstan Thomas in 1905 that an arsenical, atoxyl, is effective against trypanosome infections. It was then shown by Ehrlich & Bertheim (1907) that, contrary to an earlier belief, atoxyl consists of *p*-aminophenylarsonic acid, and there followed the well-founded theory, based on studies *in vitro* (Ehrlich, 1909a), that the activity of this substance is attributable to its reduction to the corresponding arsenoxide form.

However, Ehrlich did not urge the use of phenylarsenoxide analogues in man, believing them to be too toxic. If atoxyl, or similar substituted phenylarsonic acids, be still further reduced, beyond the arsenoxide stage, arsenobenzene derivatives are obtained, and it was among these that arsphenamine and neoarsphenamine were found. Like the arsonic acid prototype, the arsenobenzene form is believed to be relatively inactive, its parasiticidal effect coming into play only on oxidation to the corresponding arsenoxide form (Voegtlin & Smith, 1920).

In recent years (Foerster, McIntosh, Wieder, Foerster & Cooper, 1935) the idea has been revived of employing the active form, i.e. the phenylarsenoxide, in the treatment of syphilis, rather than its inactive precursors, the phenylarsonic acid or arsenobenzene forms, and *m*-amino-*p*-hydroxyphenylarsenoxide (mapharside, neohalarsine) is now very widely used. It is more toxic to the host than the other forms, weight for weight, but this is offset by its exceptional toxicity for the parasite, so that quite small doses, normally well within the range of safety for man, are efficacious. Phenylarsonic acids are not used for early syphilis, but one compound of this type, tryparsamide (see under *Trypanosomiasis*), has found an important place in the treatment of neurosyphilis, because of its powers of penetration into the central nervous system, in which location it presumably exercises its effect upon being reduced to the arsenoxide form (Hawking, Hennelly & Quastel, 1937).

Ehrlich's hope was that with arsphenamine or neoarsphenamine he might achieve his ideal of "therapia sterilisans magna"—that is eradication of infection by means of a single dose. It soon became clear, however, that for the treatment of human syphilis by these and related compounds repeated injections are necessary, preferably together with bismuth, in courses extending over many months. Such courses are, of course, exceedingly irksome, and the effect is that considerable numbers of patients do not complete their treatment. Marshall (1944), for example, states that not even half the patients who begin these long courses of injections persevere long enough to ensure a cure-rate of more than 80%. With the object, therefore, of reducing the duration of treatment to the absolute minimum, i.e. to approach as closely as possible to Ehrlich's "therapia sterilisans magna," short-term schemes of intensive therapy by intravenous drip were introduced by Chargin, Leifer & Hyman (1935). Their original technique employed neoarsphenamine during a 5-day period, but this proved to be too toxic, and considerable modifications by innumerable investigators have since been explored, including the use of mapharside instead of neoarsphenamine, rapidly-repeated injections of massive doses instead of intravenous drip, and variations in the duration of treatment.

A new era in the treatment of syphilis was inaugurated by the discovery of the activity of penicillin in spirochaetoses. The demonstration of therapeutic action against *Spirochaeta recurrentis* and *Spirillum minus* infections in mice suggested

that the substance might be effective against syphilis (Lourie & Collier, 1943), but the first cases to be actually treated were investigated in the United States (Mahoney, Arnold & Harris, 1943). The great advantages are complete freedom from serious toxic effects, and short duration of treatment such as has been aimed at by the schedules of intensive arsenotherapy, but it is too soon for final judgments to be passed.

The Chemotherapy of Trypanosomiasis

The discovery of atoxyl as a therapeutic agent against trypanosomiasis by Thomas in 1905 did not mark the beginning of arsenical therapy in these infections. Inorganic preparations, such as sodium arsenite, had been used with somewhat indifferent success since long before the recognition of trypanosomes as the cause of nagana in cattle and of sleeping sickness in man (Braid, 1858; Livingstone, 1858). After the demonstration of the activity of atoxyl, a number of Ehrlich's arsenobenzene compounds, which he synthesized as much with a view to sleeping sickness as to syphilis, were given a trial in Africa. Thus, arsphenamine and neoarsphenamine were considerably used, and one of the most promising of this series was arsenophenylglycine (Ehrlich, 1909b). A special virtue of the latter was that, unlike other arsenicals, it was effective against trypanosomes which had developed drug-resistance as a result of insufficient treatment by atoxyl, arsphenamine, or any of a considerable range of arsenicals. (The recently introduced *p*-arsenophenylbutyric acid compound of Eagle (1945) also exhibits this property, which Ehrlich (1909b) found to be shared by those arsenicals which contain an acetic acid radical.)

However, whilst most of these arsenobenzene derivatives were effective in the early stages of infection, they were eventually judged to be of little or no use once the central nervous system had become invaded. The introduction of tryparsamide by Jacobs & Heidelberger in 1919 was therefore a great step forward, since it provided a drug which is often curative in cases of cerebral involvement, although it has the disadvantage of sometimes giving rise to optic atrophy, especially in the very type of case in which its use is most called for, i.e. where the infection has reached an advanced stage.

Whilst arsenicals are, on the whole, highly efficacious against *T. gambiense*, they are of considerably less value against *T. rhodesiense*, sleeping sickness, and there has therefore always been a need of alternative remedies. One of Ehrlich's earliest triumphs in chemotherapy, directly arising from his studies of the selective affinities of living matter for dyestuffs, was the demonstration of "therapia sterilisans magna" against trypanosome infections in mice by an azo dye derivative of naphthalene and benzidine—trypan red (Ehrlich & Shiga, 1904). This did not prove to be of practical value in man or domestic animals, but it gave a lead to the production and examination of a very long series of related compounds, not all dyes, culminating in 1920 in the announcement of Bayer 205 (now known as suramin, or antrypol), a non-staining complex urea derivative.

The exceptionally high therapeutic index of this compound (Haendel & Joetten, 1920; Mayer & Zeiss, 1920) in infected mice seemed to give promise of outstanding efficacy in man. Its discovery was therefore announced with considerable fanfare, the secret of its composition was jealously guarded, and its potential value to Africa was apparently thought in some German circles to be of such a sensationally high order that Germany's forfeited colonies should be restored (Pope, 1924). With considerable ingenuity, which it was necessary to exercise no less in order to obtain the drug for analysis than in its actual examination, Fourneau, Tréfouël & Vallée (1924) elucidated and announced its composition, and this was confirmed some years later by the Germans.[1] It is now known to be of value only in early cases of sleeping sickness of both the *gambiense* and the *rhodesiense* type, and is useless after the central nervous system has become involved.

A new class of trypanocidal compounds was discovered in the aromatic diamidines. The lead to this was given by the finding of von Jancso & von Jancso (1935) and Schern & Artagaveytia-Allende (1936) that synthalin (decamethylenediguanidine) is effective against trypanosome infections in mice. Synthalin had previously been introduced into medical practice as a means of reducing the blood-sugar in diabetes, and since it was known that trypanosomes consume large amounts of glucose, the von Jancsos and Schern believed that the therapeutic effect of synthalin in trypanosome infections was mainly due to the fact that the parasites were deprived of glucose as a result of hypoglycaemia produced in the host. However, Lourie & Yorke (1937) showed that synthalin exercises an exceedingly powerful direct action on trypanosomes *in vitro*, and, moreover, insulin, which reduces the blood sugar *par excellence*, has no action on trypanosome infections. This led to the examination of a series of other guanidines, and then to related isothioureas, amines, and amidines (King, Lourie & Yorke, 1937), resulting in the eventual selection of certain aromatic diamidines, such as pentamidine, as the most promising (Lourie & Yorke, 1939). Like suramin, these compounds have proved to be highly effective in early, but not in late, *gambiense* and *rhodesiense* sleeping sickness.

The most important trypanosome infection of cattle is that caused by *T. congolense*. This is notoriously refractory to arsenicals and other compounds of proved value in human trypanosomiasis, but there is considerable promise here in the phenanthridinium series, introduced by Browning, Morgan, Robb & Walls (1938).

The Sulphonamides

Dyestuff chemistry played an important part in the inception of sulphonamide-therapy, as it had already done in the development of the chemotherapy of protozoal infections. The sulphonamido group had long been known to increase the fastness of acidic dyes to washing and to light. In 1932, Mietzsch & Klarer introduced this group to the dye chrysoidin, which had already been found by Eisenberg (1913) to exercise some antibacterial activity *in vitro* but not *in vivo*, and the resulting compound, *p*-sulphonamido-chrysoidin, or prontosil, was announced by Domagk (1935) as an effective agent against infections with haemolytic streptococci. It was then shown by Tréfouël, Tréfouël, Nitti & Bovet (1935) that the part of the prontosil molecule which conferred staining properties was in no way essential to its therapeutic powers, which were equally displayed if the substance were abbreviated to *p*-amino-benzenesulphonamide (commonly known as sulphanilamide).

The numerous sulphonamide drugs which have since been introduced into practice, selected from many hundreds synthesized towards this end, are merely substituents of sulphanilamide. In the earlier compounds, substitution was in the amino group (as in benzylsulphanilamide); these depend for their activity on being hydrolyzed in the body to

[1] [See also *BMB* 934.—ED.]

sulphanilamide. In the later and more effective compounds substitution was in the amide group (as in sulphapyridine, produced by substitution with pyridine); and they exercise their effect direct without being converted *in vivo* to sulphanilamide. Still more recently, compounds have been introduced in which substitution is both in the amino and the amide group. Such are succinylsulphathiazole (sulfasuxidine) and phthalylsulphathiazole (sulfathalidine), which have a special place in the treatment of infections of the intestinal tract, where they are slowly broken down to the effective component, sulphathiazole.

It is not possible in such a short review to mention even the names of all the more important sulphonamides, still less to describe the stages by which they have found their level in therapeutics. The crude generalization must suffice that the action of sulphanilamide and its immediate successors was found to be mainly directed against haemolytic streptococci, *B. coli* (in urinary infections), meningococci and, to a less extent, gonococci. With sulphapyridine (Whitby, 1938), pneumococci also came to be covered, and the action on gonococci was improved; and with sulphathiazole (Herrell & Brown, 1939) and sulphadiazine (Feinstone, Williams, Wolff, Huntington & Crossley, 1940), the range widened still further to include staphylococci, though the latter are not as spectacularly susceptible to any sulphonamide as they are to penicillin.

Modern Theories of Drug Action

The development of sulphonamide-therapy naturally gave a considerable impetus towards the formulating of rational conceptions of the mode of action of chemotherapeutic agents in general, including drugs other than sulphonamides. Ehrlich's theories of drug-action did recognize that the attachment of a chemotherapeutic substance to its appropriate "receptor" on the parasite cell interferes fatally, in an undefined way, with the nutritional state of that cell. Nevertheless, as McIlwain (1943b) has pointed out, these theories were conceived without relationship to contemporary developments in biochemistry. There seems to have been no attempt by Ehrlich to identify his drug-receptors with enzymes or their substrates, although much was already known which might have suggested such an identification.

The newer orientation of ideas was concisely stated by Fildes (1940) along the following lines.[2] Chemotherapeutic agents are considered to act by interfering with a metabolite essential for the parasite's growth; the drug exercises this interference-effect (i) by oxidizing a substance which requires to be reduced, or (ii) by molecular combination, forming an inactive product, or (iii) by competition for an enzyme associated with utilization of the metabolite by the parasite. For mechanism (iii), the chemotherapeutic compound would characteristically be so similar structurally to the essential metabolite that both would fit the same enzyme. In the field of general biochemistry examples had long been known of such interference with enzyme reactions, by the interposition of compounds structurally akin to the natural substrate or co-enzyme concerned, and the classical instance of this in chemotherapy was provided by Woods in 1940.

It had been known since the work of Colebrook, Buttle & O'Meara (1936) that, contrary to earlier theories, sulphonamides exercise a direct deleterious action on bacteria, without mediation of the host. It was also known, however, that this action could be antagonized by certain bacterial extracts or body-fluids (see especially Stamp, 1939; Green, 1940). Woods' outstanding contribution was to show that this antagonism can be effected specifically by *p*-aminobenzoic acid, structurally very similar to sulphanilamide, and much subsequent work has borne out his interpretation that sulphanilamide acts by blocking the enzymes which subserve to the utilization of *p*-aminobenzoic acid essential to the parasite's nutrition.

The conception has been pursued that the growth of bacteria may be inhibited by substances structurally similar to metabolites essential for the nutrition of those bacteria. For example, analogues of the essential metabolites nicotinic acid and nicotinamide have been found to be inhibitory to bacteria *in vitro* (McIlwain, 1940). The principle has been beautifully applied in the actual discovery of a new chemotherapeutic agent; it was argued *a priori* that pantoyltaurine, structurally similar to the essential metabolite pantothenic acid, should be able to exercise chemotherapeutic properties against infections of haemolytic streptococci in rats, and this was, in fact, found to be the case (McIlwain & Hawking, 1943). The chemotherapeutic effect was not of a very high order, but the value of the work is that it was a radical departure from the time-honoured technique of "hit or miss", in the search for new chemotherapeutic substances. In the past a particular chemical group may have been suspected or shown to exercise some activity against a particular infection, and the common procedure was then to synthesize hundreds—perhaps thousands—of compounds of that group, and to test each in turn, in the hope of striking upon a really useful chemotherapeutic agent. But in McIlwain & Hawking's demonstration of the chemotherapeutic effect of pantoyltaurine, the results were fairly exactly anticipated, by prior biochemical observations on both host and parasite, with the clearly-defined aim of preventing the parasite from utilizing a known metabolite essential for its existence.

[2] [See also *BMB* 929.—ED.]

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STEPS LEADING TO THE THERAPEUTIC APPLICATION OF MICROBIAL ANTAGONISMS *

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In many countries there is now proceeding extensive search for new antibiotics, investigation into the nature of their structure and biological properties, and research into the susceptibility of various infections to them. The impetus to the search has become much greater since it was shown at Oxford in 1940 that one of them—penicillin—was not only an antiseptic but a chemotherapeutic agent, that is, a drug which can be administered so as to circulate in the blood-stream in sufficient quantity to cause infecting organisms to die or at least to cease multiplication, while at the same time the body-tissues are not harmed. The work of the last few years, however, is only an expansion of studies in which, since the time of Pasteur, bacteriologists, botanists and other scientific workers have been engaged.

Although the use of the word " antibiotic " to mean the actual chemical substances involved in bacterial antagonism was only recently introduced by Waksman, Horning, Welsch & Woodruff (1942), the observation of antagonisms between bacteria, and even the idea that one bacterium might thus be used to combat another, has frequently occurred. Vuillemin wrote in 1889 :

" Nul n'a traité de parasite le lion qui fond sur sa proie, ou le serpent qui instille du venin dans la plaie de sa victime avant de la dévorer. Ici, point d'équivoque : un être détruit la vie d'un autre être pour entretenir la sienne : le premier est complètement actif, le second est complètement passif : celui-là est sans restriction contraire à la vie de celui-ci. Le cas est si simple qu'on n'a jamais songé à lui donner un nom. Cette condition au lieu de s'offrir isolément à notre examin, peut se présenter comme facteur de phénomènes plus complexes. Pour simplifier le langage, nous l'appellerons alors *antibiose* ; l'individu actif sera l'*antibiote* ; l'individu passif sera le *support*."

" Antibiosis " finally came to mean the interference with the growth of one micro-organism by another. Thus we arrive at the modern word " antibiotics " as the name for the chemical substances, products of metabolism, which are frequently involved in this antagonism. But long before the adoption of these terms, people of Central Europe, the Ukraine, Central America, and possibly of other countries, deliberately grew moulds, probably of the kinds called *Penicillia*, to be used as applications to wounds. The taking of yeast was advocated in 1852 by an English practitioner, Mosse, for the treatment of boils—a treatment which had already been used in the north of France, and is even now still in use ; but as the yeasts have never been clearly shown to produce any definite chemical substance combating pathogenic organisms, any beneficial effect from them is possibly caused by their vitamins.

First Scientific Observations

The first demonstration of antibiotic action against a pathogenic organism was made by Pasteur and his colleague Joubert in 1877. They observed that if a culture of anthrax

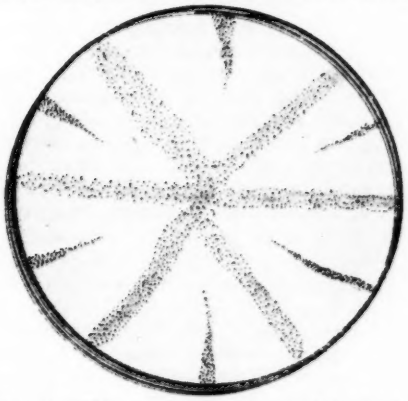
* Based on the Lister Memorial Lecture, which was delivered at the Royal College of Surgeons on 11 October 1945 and published in the *British Medical Journal*, 1945, **2**, 635-642. The present paper contains additional material from several subsequent lectures.

bacilli in urine was contaminated by organisms from the air the anthrax bacilli were destroyed :

" L'urine, ai-je dit, neutre ou légèrement alcaline, est un excellent terrain de culture pour la bactéridie ; que l'urine soit pure et la bactéridie pure, et dans l'intervalle de quelques heures, celle-ci est tellement multipliée que les longs filaments qui la composent remplissent le liquide d'un feutrage d'aspect cotonneux ; mais si, au moment de déposer dans l'urine les bactéridies à titre de semence, on sème en outre un organisme aérobie, par exemple une des bactéries communes, la bactéridie charbonneuse ne se développe pas ou très-peu, et elle périt entièrement après un temps plus ou moins long. Chose bien remarquable, ce même phénomène se passe dans le corps des animaux qui sont le plus aptes à contracter le charbon et l'on arrive à ce résultat surprenant qu'on peut introduire à profusion dans un animal la bactéridie charbonneuse sans que celui-ci contracte le charbon ; il suffit qu'au liquide qui tient en suspension la bactéridie on ait associé en même temps des bactéries communes. Tous ces faits autorisent peut-être les plus grandes espérances au point de vue thérapeutique."

Pasteur and Joubert thus found that by introducing some common bacteria into the animal body at the same time as the anthrax bacillus, the death of the animal from anthrax could sometimes be prevented. As Pasteur was at the time mainly interested in immunity, he did not pursue the matter. In 1885, Babès demonstrated on solid as well as in liquid media that one organism can elaborate a substance which will stop the growth of another, and suggested that " L'étude continuée et généralisée de cette action réciproque des bactéries les unes vis-à-vis des autres, pourra conduire à des données thérapeutiques." He correctly deduced from his experiments that the inhibitions of bacterial growth he had observed were brought about by a definite chemical substance produced by the antagonistic organism. In the same year Cantani (1885) evolved the idea of replacing a harmful

FIG. 1. A METHOD, WHICH IS STILL IN USE, OF DETECTING BACTERIAL ANTAGONISM (Frost, 1904)



Frost, writing in 1904, gave this illustration of the method, which had first been described by Garré in 1887. *Ps. fluorescens* was planted on the surface of an agar plate in 6 radial streaks and the plate was incubated for 24 hours, to give time for metabolites to be produced and to diffuse through the agar. *B. typhosus* was then planted in radial streaks alternating with those already grown. After further incubation it was apparent that *Ps. fluorescens* had produced an inhibitor which had prevented the *B. typhosus* from growing, except at the periphery of the plate, where the streaks were furthest apart.

FIG. 2. A METHOD OF TESTING ANTAGONISM BETWEEN BACTERIA IN FLUID CULTURE MEDIA (Frost, 1904)

The effect of one fluid culture on another was tested by separating the cultures by a substance permeable to metabolites but not to bacteria. Previously this had been done by putting one culture inside and the other outside a Pasteur-Chamberland filter. Frost modified the method by enclosing one culture in a collodion sac and suspending the sac in a flask containing the other culture. In the experiment which he illustrated, the sac contained *B. typhosus* and the flask the opposing organisms. Certain unidentified soil bacteria killed 98% of the *B. typhosus* in 6 days.

organism (the tubercle bacillus) in the tissues of the lungs with an ill-defined harmless organism (which was probably a mixture of species) called *Bacterium termo*. He treated a tuberculous patient by insufflating large amounts of cultures of this bacillus into the lungs, and claimed good results. This was the first example of the idea of the replacement of one pathogenic organism either by another less pathogenic or, if possible, by an innocuous organism, an idea which frequently recurs in subsequent pathological and clinical literature.

Two years later the Swiss, Garré (1887), introduced a method for examining bacterial antagonism by means of alternating streaks of two species of bacteria grown on a Petri dish containing agar—a method essentially the same as some of those still in use. Garré wrote :

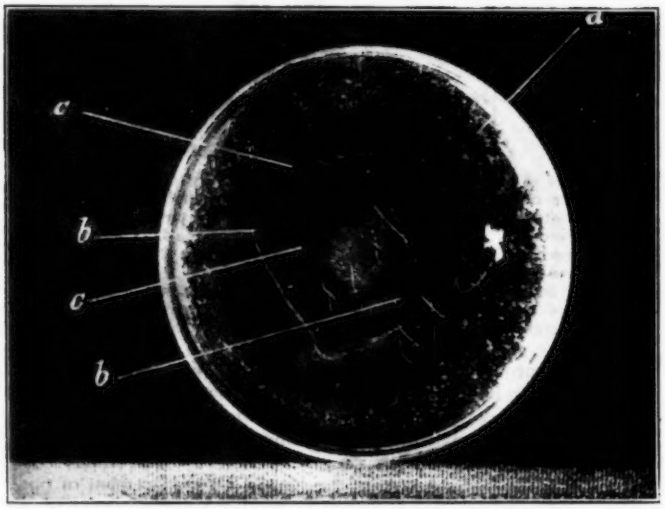
" Auf die brach erkaltete Platte impfe ich gleichzeitig in parallelen Strichen alternirend den Bac. fluorescens und den Staph. pyog.—so zwar, dass jeweilen die Distanz der Impfstriche grösser wird, von 3-15 mm. Der Bac. fluoresc. wächst rascher ; seine Ausscheidungsproducte diffundiren in die Umgebung und werden für die näher gelegenen Staphylococcusimpfungen zum absoluten Entwicklungshinderniss. . . . Es handelt sich also nicht um ein Ueberwuchern und Verdrängen der einen Art durch die andere, rascher sich ausbreitende,—wie etwa in einem Garten üppiges Unkraut zarte Pflänzlinge ersticken kann. Es ist auch kein

Vorwegzehren der disponibeln Nahrung durch rascher keimende Mikroorganismen, sondern es liegt hier ein Antagonismus durch Ausscheidung specifischer, leicht diffundirender Stoffe vor, die für die einen Arten ein Entwicklungshinderniss bilden, für die andern aber durchaus indifferent sind."[1]

Garré's paper was not illustrated, but in 1904 Frost described seven methods for the investigation of the antagonistic properties of bacteria, and among these was Garré's method, of which Frost published an illustration (Fig. 1). One of the most interesting methods described by Frost is that in which he grew one bacterium in liquid medium in a flask and another in medium contained in a collodion sac suspended in the flask; an antibiotic produced by the bacterium in one medium could diffuse through the collodion and inhibit the bacterium in the other (Fig. 2).

The first photographic record of the phenomenon of antibiosis was published in 1889 at Kiel in a thesis by Doehle (Fig. 3). This illustration—the photograph for which was actually taken by Hoppe-Seyler himself—shows the anthrax bacillus, which had been sown in a solid medium, being inhibited in the neighbourhood of a certain micrococcus, which Doehle had isolated and called *Micrococcus anthracotoxicus*, planted in the form of a square.

FIG. 3. THE FIRST ILLUSTRATION OF ANTIBIOTIC ACTION (Doehle, 1889)



Doehle poured a plate of gelatin medium containing anthrax bacilli. On the surface he planted, in a square, a coccus to which he gave the name *Micrococcus anthracotoxicus*. After incubation the anthrax bacilli had grown in the peripheral part of the plate (a), but both outside and inside the square formed by the *Micrococcus* (b) the anthrax bacilli had failed to grow (c).

Thus, by 1889 the phenomenon of microbial antagonism was well known and had been illustrated, and not only Pasteur, but nearly all the bacteriologists who worked on it at that time, had in mind its use for therapeutic purposes. Emmerich, for instance, in 1887 had shown that some effect could be obtained in protecting rabbits from experimental anthrax by the simultaneous injection of a streptococcus from a case of erysipelas. Clinicians had long believed that an attack of erysipelas was beneficial in certain chronic infections, and in 1883 Fehleisen had deliberately treated a case of lupus by injecting streptococci from a patient with erysipelas. Emmerich, however, demonstrated that there was no antagonism between the two organisms *in vitro* on the surface of a solid medium, so he ascribed his results to stimulation by the cocci of the body-cells, which were then able to demolish the bacilli.

Pyocyanase and its Clinical Utilization

The most promising attempt at the therapeutic use of a natural antibacterial substance was started by the work of Bouchard in 1889. He observed that the injection of a culture of *Ps. pyocyanea* could confer some degree of protection on rabbits infected at the same time with anthrax. Metabolic products of the bacteria were next used instead of whole cultures, and trials on man of "proteins" from cultures of *Ps. pyocyanea* were made ten years later by Honl & Bukovsky (1899).

Meanwhile, Emmerich and Löw were independently working with the same organism, and in 1899 they prepared from old cultures of *Ps. pyocyanea* an extract which they called pyocyanase, capable of lysing suspensions of *B. anthracis in vitro*, and also bactericidal to *Bact. typhosum*, *C. diphtheriae*, staphylococcus, and *P. pestis*. At first they attempted systemic administration of pyocyanase in experimental infections in animals, but it proved too toxic and eventually was used only as a local application to infected parts. Its clinical use for many infections by local application was widespread for some time, but by about 1912 or 1913 had practically ceased, apparently because the commercial preparations made at that time were inactive. Although Emmerich and Löw seem to have confused the antibiotic effect of the substance with the views on immunity held at that time, the work with pyocyanase was the first serious attempt to use a bacterial product or antibiotic in medicine for curative purposes.

The important idea of differential toxicity, which so largely governs the choice of antiseptics today, must have been widespread at that time, for Escherich wrote in 1906:

"Die Wiederaufnahme dieser Bestrebungen wurde erst möglich, als die Fortschritte der Wissenschaft uns mit Stoffen bekannt machten, welche das Vermögen einer hohen bacteriziden Fähigkeit besassen, ohne die den bisherigen Antiseptizis anhaftenden Schädigungen der Gewebe. Es sind dies die auf dem Wege der Autolyse aus Bakterien gewonnenen bakteriziden Substanzen, auf deren Vorkommen und Bedeutung zuerst Emmerich und Löw die Aufmerksamkeit gelenkt haben."[1]

[1] "I inoculated on the untouched cooled [gelatin] plate alternate parallel strokes of *B. fluorescens* and *Staph. pyogenes*. This was carried out so that the distance between the inoculated strokes increased from 3 to 15 mm. *B. fluorescens* grew more quickly. Its products of secretion diffused into the surroundings and were completely inhibitory for the near-by staphylococcal inoculation. . . . Thus it is not a question of overgrowth or crowding out of one by another quicker-growing species, as in a garden where luxuriantly growing weeds kill the delicate plants. Nor is it due to the utilization of the available foodstuff by the more quickly growing organisms; rather there is an antagonism caused by the secretion of specific, easily diffusible substances which are inhibitory to the growth of some species but completely ineffective against others."

[1] "The resumption of these endeavours first became possible when the march of science made known to us substances which possessed a high bactericidal capacity without at the same time harming the tissues as do previously known antiseptics. These are the bactericidal substances obtained from the autolysis of bacteria, to the existence and significance of which Emmerich and Löw first drew attention."

It is interesting to note that as recently as 1943 Rake, Jones & McKee found that a good preparation of pyocyanase inhibited the growth of *Str. pyogenes* at a dilution of 1 in 24,000. Recently Doisy and his colleagues (Hays, Wells, Katzman, Cain, Jacobs, Thayer, Doisy, Gaby, Roberts, Muir, Carroll, Jones & Wade, 1945) have isolated from the products of *Ps. pyocyanea* four crystalline substances, Pyo I, II, III, and IV, which act powerfully against gram-positive organisms and appear to have little toxicity to animal tissues.

Antibiotics from a Micrococcus and from Aerobic Sporing Bacilli

While these clinical trials were being made, other investigations were being recorded. In 1903 Lode wrote of an accidental contaminant, a gram-positive coccus, which he had found while preparing a plate of *Micrococcus tetragenus* for class demonstration (Fig. 4). He subcultured this organism and showed that it produced a diffusible substance which strongly inhibited the growth of anthrax bacilli and *Staph. aureus*, but not *Bact. coli* or Friedländer's bacillus. Lode did comprehensive and painstaking investigations of this substance: he showed that it was bactericidal, and that it was not an enzyme, though it was thermolabile, being inactivated slowly by heat. It could be dried by vacuum distillation and was soluble in alcohol but not in ether. Neither the micro-organism nor its metabolic products were toxic to animals, but unfortunately in artificially produced infections in mice the product had no chemotherapeutic effect.

In 1907 a Frenchman, Nicolle, demonstrated the existence of bactericidal substances produced by a common spore-forming soil bacterium known as *Bacillus subtilis*. At about the same time, in France, Rappin experimented upon the effects of filtrates of the spore-forming *B. subtilis*, *B. mesentericus*, and *B. megatherium* on the tubercle bacillus. He wrote in 1912:

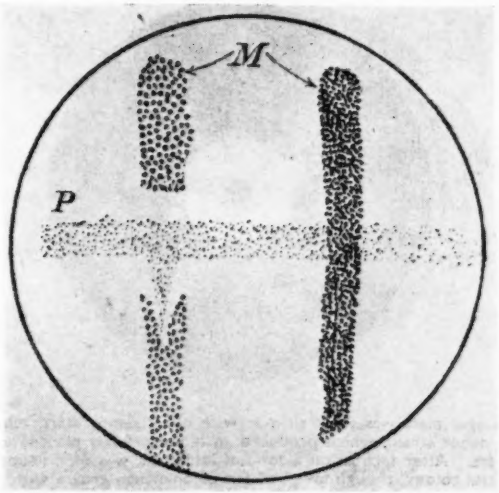
"Après avoir constaté, il y a déjà longtemps, l'action si énergique de ces diastases, je fus naturellement amené à penser que, peut-être, elles étaient susceptibles aussi de posséder des propriétés importantes sur l'évolution de la tuberculose et il y a près de deux ans, j'instituai quelques expériences dans le but de vérifier cette hypothèse. J'injectai ainsi à quelques cobayes préalablement inoculés de tuberculose des cultures filtrées de *B. mesentericus* et je possède encore aujourd'hui un animal de cette première série qui, traité de la sorte, conserve plus de vingt et un mois après l'inoculation virulente toutes les apparences d'une parfaite santé. Un autre qui mourut au bout de neuf mois, sans avoir manifesté aucun trouble et après avoir suivi une ascension regulière de son poids ne présentait aucune lésion à l'autopsie: on notait seulement l'existence d'un ganglion à l'inoculation et un autre de l'autre côté. Enfin, fait très important, le produit de raclage de ces ganglions inoculé à un autre animal n'a paru déterminer jusqu'ici chez ce dernier aucune réaction.

"Répétée sur d'autres cobayes, cette expérience a donné des résultats semblables, et je possède plusieurs animaux

FIG. 4. ONE OF THE FIRST BACTERIAL ANTAGONISMS STUDIED (Lode, 1903)

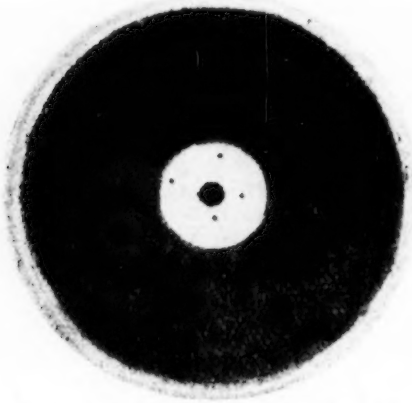
Lode noticed that a culture of *Micrococcus tetragenus* was inhibited by an accidental contaminant—a coccus. He isolated this coccus and studied its antagonistic properties extensively. He found that some kinds of bacteria were inhibited more than others, and some not at all (differential action), that the number of bacteria to be inhibited affected the efficiency of the antagonist (inoculum-size effect), and that the antagonist was bactericidal. He succeeded in obtaining the active material in fluid culture and made observations on the conditions necessary for its production and on its chemical nature.
The gelatin plate illustrated was sown with *M. tetragenus*, which was inhibited round the three colonies of the coccus.

FIG. 5. INHIBITION BY PNEUMOCOCCUS OF MENINGOCOCCUS (Colebrook, 1915)



Colebrook noticed that the pneumococcus inhibited many other bacteria in culture. His illustration shows a streak of the pneumococcus (horizontal) crossed by two streaks of meningococcus (vertical). The pneumococcus inhibited the left-hand streak, which was made by inoculating a relatively small number of meningococci, but had no effect on the right-hand streak, where the inoculum was heavier. Most antiseptic substances are subject in some degree to this "inoculum-size effect", and if the effect is great the therapeutic value of the substance is thereby reduced.
Colebrook attempted to use the antibiotic properties of the pneumococcus by spraying it into the throats of carriers of the meningococcus, in the hope that it would establish itself and replace the meningococcus.

FIG. 6. INHIBITION OF ONE STRAIN OF BACT. COLI BY ANOTHER (Gratia, 1925)



The agar plate was sown all over with one strain of *Bact. coli*, and the other strain, which produced an inhibitor, was planted in the centre. After incubation a zone of inhibition was seen round the central colony, though four isolated colonies had grown within the zone. It is not uncommon for a few resistant bacteria to grow from a strain otherwise sensitive to an antibiotic and this may complicate testing, particularly in liquid media.

traités de la sorte à des époques plus ou moins éloignées de l'inoculation tuberculeuse qui demeurent en pleine santé apparente, alors que leurs témoins sont morts présentant toutes les lésions classiques de la tuberculose expérimentale.

" Pour obtenir ces résultats, un nombre relativement faible d'injections a suffi. Quelques-uns de ces animaux ont reçu seulement deux injections de bouillon filtré de *mesentericus* à la dose de un à deux centimètres cubes sous la peau et d'autres la même quantité mais dans la cavité péritonéale, sans que ni les uns ni les autres n'aient du reste paru touchés par ces injections, ni présenté de réactions."

Zukerman & Minkewitsch (1925) found on a plate of pseudo-diphtheria bacilli an accidental contaminant antagonistic to their growth. The organism was *B. mesentericus vulgatus*, and it showed antagonism only to diphtheria and pseudo-diphtheria bacilli, not to staphylococci, *Bact. coli*, *Bact. typhosum*, or a number of dysentery bacilli. Also in 1925 Rosenthal contributed work on the bacteriolytic powers of *B. scaber*, another spore-forming organism. Some of the more recent work with spore-forming organisms will be referred to later.

Bacterial Substitution Therapy

There were various attempts after Cantani's to replace harmful by harmless bacteria. Schiøtz (1909), a Danish physician, noticed that a patient with a staphylococcal infection, wrongly diagnosed as having diphtheria and placed in a diphtheria ward, did not develop the disease. Schiøtz then deliberately sprayed suspensions of staphylococci into the throats of carriers with, he claimed, good results. The best-known attempt at such " replacement therapy " was that suggested by Metchnikoff (1909), to replace what he considered harmful organisms in the intestine by the Bulgarian lacto-bacillus ; later workers used a similar organism, *B. acidophilus*, in the same way. In 1915 Newman treated cases of cystitis by injecting lactic acid bacilli into the bladder ; in the same year Colebrook showed inhibition of meningococci by pneumococci (Fig. 5), and suggested using the pneumococcus to combat the meningococcus in the throats of carriers ; and in the following year Nissle (1916), maintaining that certain strains of *Bact. coli* were antagonistic to other, more harmful organisms, put on the market a product called " mutaflor ", a preparation of living *Bact. coli* which were supposed to replace these more harmful organisms in many intestinal diseases. A similar antagonism was described in 1925 by Gratia, who published an illustration demonstrating the inhibition of one strain of *Bact. coli* by another (Fig. 6). In the same year Alivisatos showed the inhibition of staphylococci by pneumococci (1925 ; Fig. 7). The pneumobacillus of Friedländer was suggested by Papacostas and Gaté (Papacostas & Gaté, 1921 ; Gaté & Papacostas, 1921) for the treatment of diphtheria carriers.

In connexion with the idea of " fighting " one bacterium with another, an interesting line of reasoning was displayed by a Pole, Nitsch (1908), who was in France at a time when the towns of Lyons and Versailles were notorious for their freedom from epidemics of cholera. It occurred to him that possibly bacteria in the air of these regions might have the property of inhibiting the growth of the cholera organism, and that if they were swallowed they might exert their antagonistic effects in the intestine. He found eleven strains of air bacteria out of 220 from various parts of Versailles, and four out of 253 from Paris, which when cultured at

FIG. 7. INHIBITION OF STAPHYLOCOCCUS BY PNEUMOCOCCUS (Alivisatos, 1925)

The culture plate was sown with a suitable mixture of pneumococcus and staphylococcus. After incubation, clear zones where the staphylococcus had not grown were seen round the colonies of pneumococcus. Alivisatos tried to show whether the inhibition was caused by a chemical metabolite by growing the pneumococcus in ascites broth and spreading the broth, after sterilizing it by filtration, on a plate sown with staphylococci, but the result of this experiment was negative.
He showed that many strains of pneumococcus had inhibitory power.

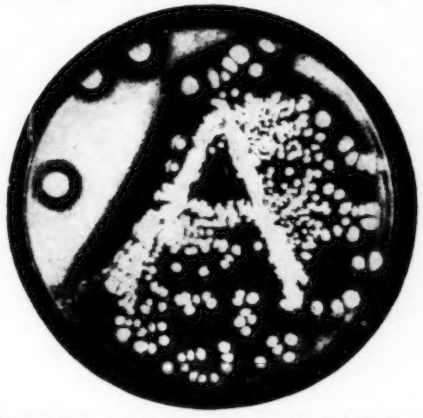
37° C. inhibited the growth of *Vibrio cholerae*. Another Pole, Choukevitch, in 1911 continued these observations, with three of Nitsch's Versailles strains of cocci. He confirmed that if a streak of one of these organisms was planted on an agar surface and *V. cholerae* was planted at the same hour on the following day, growth of the vibrios did not take place within a distance of 1 cm. from the antagonist. He found that the inhibition was not due to acid, that the inhibitory substance developed in broth as well as in solid media, and that it was thermolabile and not capable of filtration. He attempted to establish the cocci in the intestines of new-born rabbits, but was not able to demonstrate any protection against ingested cholera organisms.

Other Ideas for Use and Production

In 1917, in Australia, Greig-Smith made the first observations on the actinomycetes ; he noticed that some of these organisms grown from soil had the power of stopping the growth of certain bacteria. In 1921 Lieske published a monograph on the subject of antibiotics produced by actinomyces and there were important contributions soon afterwards by Gratia & Dath (1924, 1925), who examined many sources for organisms producing antagonistic substances, and were particularly struck by the effects of a streptothrix which caused the dissolution of the staphylococci with which the plate was heavily sown (Gratia, 1934 ; Fig. 8). They used the lytic powers of the substance produced by their streptothrix or actinomycete instead of heat to kill the bacteria used in the preparation of vaccines known as mycolysates. Kimmelstiel, Much and Sartorius (Kimmelstiel, 1923, 1924 ; Much, 1925 ; Much & Sartorius, 1924 ; Sartorius, 1924) made similar attempts to use bacteria dissolved by ferments produced by micro-organisms ; their product was sold under the name of " sentocym ". More recent work on *Actinomyces* will be referred to later.

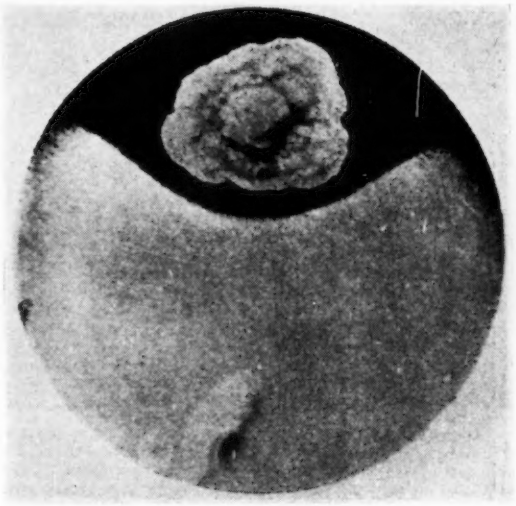
In the nineteen-twenties a new idea, which has yet to be developed, was introduced by Schiller (1924a, 1924b, 1925a, 1925b, 1927 ; Schiller & Giltscher, 1928). He brought forward evidence that yeasts and possibly other organisms could be trained to produce substances that were antagonistic to certain bacteria, by growing them on media in which the only source of nitrogen was the bodies of the bacteria it was desired to antagonize. He claimed that in such experiments yeasts developed a thermolabile inhibitory substance which might be of value in therapeutics. He called the phenomenon " induced " antagonism, in contradistinction to the " natural " antagonisms.

FIG. 8. INHIBITION BY A STREPTOTHRIX (Gratia, 1934)



Gratia showed that some strains of streptothrix had the power to dissolve bacteria. A heavy emulsion of staphylococci was incorporated in a gelatin culture plate and the streptothrix was planted on the surface (A and isolated colonies). After 3 days, as the illustration shows, the staphylococci near the streptothrix had dissolved and in a week the medium had cleared completely. Gratia made use of a streptothrix to dissolve bacteria for vaccines, and the " mycolysates ", as he called them, were used clinically.

FIG. 9. INHIBITION OF ONE MOULD BY ANOTHER (Harder, 1911)



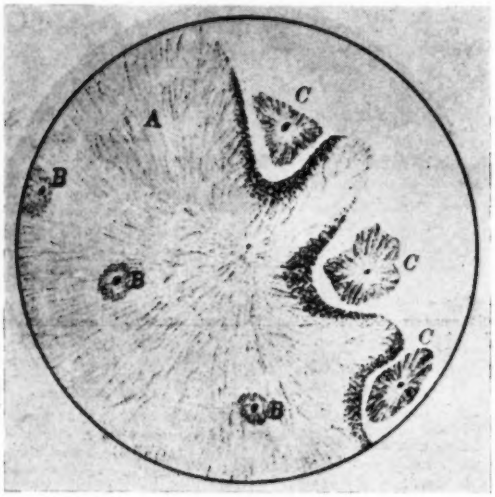
Harder, a botanist, published in 1911 an account of inhibitions which he had observed among Basidiomycetes and Ascomycetes growing in culture. This illustration from his paper shows a colony of *Penicillium luteum* (above) stopping at a distance the spread of *Stereum purpureum* (below) over the culture plate.

Mould Products

The first example of a mould yielding an antibiotic was recorded in 1896 by Gosio, when he obtained a crystalline substance from a *Penicillium* in the course of his investigations on mouldy maize, thought at that time to be the cause of pellagra. The antibiotic is now called mycophenolic acid. Gosio showed that the crystalline material inhibited the growth of anthrax bacilli, and regretted that he was unable to perform animal experiments because of lack of material. Recently the antibiotic and biological properties of this substance have been more fully investigated (Florey, Gilliver, Jennings & Sanders, 1946). Vaudremer found that the mould *Aspergillus fumigatus* would " digest " the tubercle bacillus, though he had a dispute with Rappin over the question of priority. In 1913 Vaudremer reported animal experiments, and described in the following terms the results of injecting *A. fumigatus* filtrates into patients suffering from tuberculosis :

" Toute conclusion est également prématurée si on envisage le traitement de la tuberculose humaine. *Depuis 1910, nous avons traité plus de deux cents malades* avec les extraits

FIG. 10. PRODUCTION OF AN ANTIBIOTIC BY ONE SPECIES OF PENICILLIUM BUT NOT BY ANOTHER (Porter, 1924)



Porter, a botanist, tried to protect plants from attack by fungi, by using the antibiotic properties of bacteria or of other fungi. He tried both growing the inhibitory organism near the plant it was desired to protect, and also injecting its metabolic products, e.g. into an orange exposed to the spores of a mould. His illustration shows that one species of *Penicillium* (C) inhibited at a distance the growth of *Pestalozzia* (A), while another species of *Penicillium* (B) had no effect.

d'*A. fumigatus* ; le traitement a été appliqué dans plusieurs hôpitaux et sanatoriums parisiens. Des faits que nous avons observés, on peut conclure à l'innocuité de ces injections qui ne provoquent jamais de réaction fébrile. Parfois même on voit survenir des guérisons inespérées. Dans d'autres cas, on observe une amélioration passagère ; mais malheureusement les faits sont encore nombreux où la tuberculose poursuit son évolution."

Vaudremer had been trying to get a direct effect on the tubercle bacillus *in vivo* by injecting a mould metabolism solution. It is now known that *A. fumigatus* produces four antibiotic substances, at least one of which, helvolic acid (Chain, Florey, Jennings & Williams, 1943 ; Waksman & Geiger, 1944) has some action against the tubercle bacillus *in vitro* (Jennings, 1945).

Meanwhile, during this quarter-century of sporadic development, important methods and observations had been elaborated by botanists. Reinhardt, for example, in 1892, had described the inhibition of a fungus, *Peziza trifoliorum*, by a bacterium at a distance of 25 mm., as well as antagonisms between *Penicillia* and *Aspergilli* when these were grown on solid media. In 1911 Harder showed the inhibition of *Stereum purpureum* by *Penicillium luteum* (Fig. 9). Many observations were made and general technical procedures furnished by Porter (1924), who noted, among other things, that a penicillium inhibited the growth of *Pestalozzia* (Fig. 10). An interesting example of fungal inhibition, using the technique of growth on agar, was described by Nadson & Jolkevitch (1923), who found a contaminating fungus, which they later called *Spicaria purpurogenes*, with the capacity to kill the common baker's yeast.

The observation on the moulds which has had most consequence was made in 1929, when Fleming noticed a mould which had accidentally fallen on to one of his culture plates and which was causing the dissolution of staphylococci in its neighbourhood (Fig. 11). He subcultured this mould (subsequently identified as *Penicillium notatum*) in broth and found that it secreted something into the broth which had the power of stopping the growth and eventually slowly killing many pathogenic organisms. He called the broth in which this antibacterial substance had been secreted penicillin, and later the antibacterial substance itself was given this name. He observed that the broth acted on many important pathogenic organisms such as the streptococcus and staphylococcus, while others, such as *H. influenzae* and the Salmonellas, remained unaffected. The crude metabolism liquid was shown not to be more toxic to animals than broth in which nothing had grown. No curative experiments were performed on experimental infection in animals, but the suggestion was made that the substance would be useful for dressing septic lesions in man, and indeed some were so treated without, however, any very striking results. The clinical possibilities of this antibiotic were not pursued until the beginning of the next decade, although Fleming maintained his strain of the mould and made use of the broth in differential culture media in the laboratory.

The Position in 1939

The position at the end of the nineteen-thirties may perhaps be summarized thus. A very large number of observations on antibiosis had been recorded from 1877 onwards—some of the most striking have been considered, but there were a great many more—and from all this work

FIG. 11. THE FIRST OBSERVATION ON PENICILLIUM NOTATUM (Fleming, 1929)

This is a photograph of the original plate on which Fleming found a colony of *Penicillium* dissolving the surrounding colonies of staphylococci. Not all bacteria were susceptible to the action of the mould, and Fleming made use of this property by incorporating broth on which the mould had grown in media for differential culture. Among other experiments he showed that the broth was more toxic to bacteria than to human leucocytes *in vitro*.

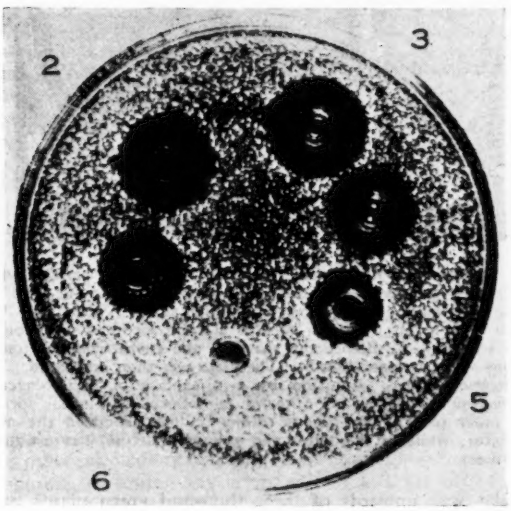
certain proposals had emerged for the use of micro-organisms in therapeutics :

1. The replacement of a pathogenic organism by another and less harmful organism—for example, "*Bact. termo*" to replace the tubercle bacillus in the lungs ; staphylococci to replace diphtheria bacilli, and pneumococci to replace meningococci, in the throat ; lactic acid bacilli and *Bact. coli* to replace organisms infecting the intestine.
2. Artificial immunization by one organism to protect against infection by another.
3. The use of lytic substances from one organism for the preparation of soluble vaccines of other species—for example, Much's sentocym and Gratia's mycolysates.
4. The use of soluble bacterial or mould products as a topical application for the treatment of local infections—for example, pyocyanase and penicillin broth.
5. The use of soluble bacterial or mould products by parenteral injection to treat established diseases—for example, pyocyanase in anthrax, and Vaudremer's extracts of *Aspergillus fumigatus* and Rappin's extracts of *B. subtilis* in tuberculosis. These were examples of true chemotherapeutic use.

Discovery of Penicillin's Chemotherapeutic Powers

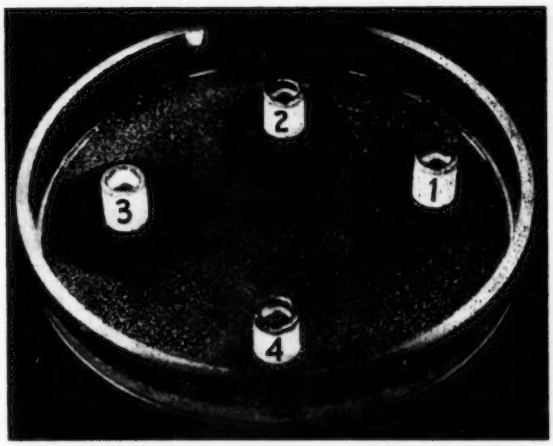
In the early part of 1939 a wide investigation of natural antibacterial substances was planned by Dr. Chain and myself, and penicillin was one of the first chosen for investigation. In 1932 Clutterbuck, Lovell & Raistrick had published

FIG. 12. ACTIVITY OF LICHENS AGAINST BACTERIA (Burkholder, Evans, McVeigh & Thornton, 1944)



Extracts of lichens were made in a mortar and tested by the cylinder-plate method against various bacteria. In the experiment illustrated the lichens were extracted by grinding them with ether ; the ether was then evaporated and the residue taken up in buffer, pH 7·4, for testing. This is a useful method for testing for the presence of antibiotics which are insoluble in water but can be extracted by an organic solvent.
The bacterium on the plate is *B. cereus* and the numbered cylinders contain extracts of *Cladonia cristatella* (2), *C. caroliniana* (3), *C. Grayi* (5) and *C. coniocraea* (6).

FIG. 13. INHIBITION BY GREEN PLANTS (Osborn, 1945)



Plants have from prehistoric times been used for medicines and some have had a high reputation for preventing suppuration in wounds. Certainly many plants can be shown to contain antibacterial substances. The cylinders on the plate illustrated contain watery extracts of :

1. *Allium sativum*
2. *Spiraea aruncus*
3. *Ranunculus bulbosus*
4. *Prunella vulgaris*

The last, which was negative in this test, was called "self-heal" and was recommended by 17th century herbals for treating wounds.

a paper in which they showed that penicillin could be produced by the mould on a synthetic medium, and that it could be extracted from water into ether if the water was made acid, but that large losses of antibacterial activity occurred on the evaporation of the ether. They had recognized that penicillin was most stable around neutrality, but had stated that it was a very labile substance, and they did not carry the work further. Based on this work we found, with our colleagues at Oxford, a method for extracting the penicillin and making preparations which, though crude, had high antibacterial activity. After some preliminary biological experiments with these extracts, it became clear that the substance might be of exceptional interest and a team of workers was assembled in the Sir William Dunn School of Pathology at Oxford. In 1940 the first observations on penicillin from Oxford were published (Chain, Florey, Gardner, Heatley Jennings, Orr-Ewing & Sanders), which clearly distinguished this antibiotic from any of its predecessors. They showed that artificial infections in mice with the streptococcus, staphylococcus and *Cl. septique* could be completely controlled by injections of a penicillin extract, so that there was almost 100% survival from infections which would otherwise certainly have been fatal. In other words, penicillin was a chemotherapeutic drug of great power, that is to say, it was of such low toxicity that it could, without producing any toxic signs, be injected by parenteral routes in amounts which would stop the growth of bacteria in all parts of the body. Many other investigations were made. A full account of these, together with the results of the first clinical trials, was published in 1941 (Abraham, Chain, Fletcher, Florey, Gardner, Heatley & Jennings), and the results of a larger clinical trial in 1943 (Florey & Florey). Two of the facts of

especial importance which emerged from the laboratory work at Oxford were, firstly, that extracted penicillin was fully active in the presence of pus and tissue-breakdown products, and of large numbers of bacteria, all of which impaired the antibacterial action of all the sulphonamides known at that time, and, secondly, that even very impure extracts were non-toxic to animals in concentrations hundreds of times greater than those required to inhibit bacteria, and that with increasing purity of the extracts the toxicity became still less (Florey & Jennings, 1942).

Examples of Recent Work on Some Special Groups

Sporing Aerobic Bacilli

At the present time a widespread survey of natural sources, such as plants, moulds, and bacteria, is being made for the presence of antibiotics. In 1939 Dubos, after long study and preparation of soil bacteria, had described the isolation of a powerful selective antibiotic from a spore-forming soil bacillus, *B. brevis*. In collaboration with others he investigated this substance, now known as tyrothricin, from both a chemical and biological point of view, and in the clinic. Tyrothricin was found to consist of two crystalline polypeptides, gramicidin and tyrocidine, which are both antibiotics. They have proved too toxic to act as chemotherapeutic agents, but have had some use as local applications, and have proved to be of the greatest interest to the crystallographers for the study of protein structure by *x*-ray methods. Gramicidin was shown to be particularly active against gram-positive organisms such as pneumococci and streptococci.

From the group of spore-forming bacteria, also, Johnson and his collaborators (Johnson, Anker & Meleney, 1945) have prepared a substance which they have named "bacitracin". This seems to affect the same range of bacteria as penicillin; it has been shown capable of controlling experimental disease in mice, and can be used successfully at least as a surface application for disease in man.

This group of organisms is the subject of active research at the present time in more than one country, and new publications continue to appear.

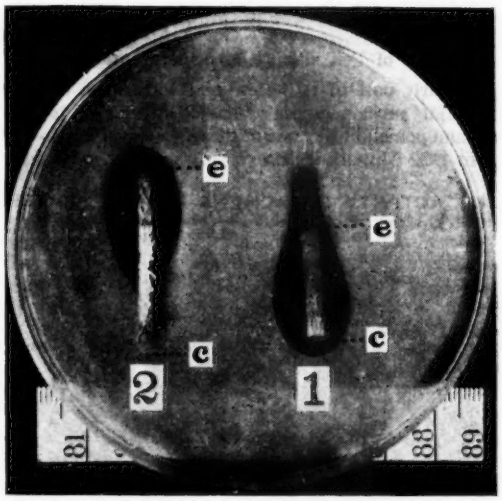
Actinomycetes

Among several antibiotic substances extracted by Waksman and his colleagues (Schatz, Bugie & Waksman, 1944) from the actinomycetes, one known as streptomycin has been investigated which has proved to be particularly interesting. Although little toxic to animal tissues, it stops the growth of many pathogenic organisms at high dilution, and is active against some of the organisms not inhibited by penicillin, for instance, some of the gram-negative organisms such as *Bact. coli* and *Ps. pyocyanea*, and it has some effect against the tubercle bacillus. Its possible use for the treatment of tuberculosis is now being actively pursued, but it is still too early to say what it may achieve. Waksman & Woodruff (1942) have also described streptothricin, a substance similar to, but more toxic than, streptomycin; and Gardner & Chain (1942) isolated, from an accidental contaminant, proactinomycin, which acts against gram-positive organisms, and has been shown to have chemotherapeutic properties against peritoneal infection by the streptococcus when given by mouth (Florey, Jennings & Sanders, 1945). Its range of antibacterial activity is very similar to that of penicillin, but it is probably too toxic to be used in man, and such great progress has been made in chemotherapy during the last few years that it is now of little interest except to add to the list of naturally-produced antibacterial substances with chemotherapeutic properties.

Higher Plants

Higher forms of plant life also contain antibacterial substances. Burkholder and his colleagues (Burkholder, Evans, McVeigh & Thornton, 1944) examined a large number of lichens, which are a symbiosis of fungus and alga, by grinding them up with saline or other solutions and testing the liquid extract, and found that many contained inhibitory substances (Fig. 12), some of which it was suggested might be lichen acids, but as yet no real chemical examinations are reported and no further biological examinations. In a

FIG. 14. METHOD OF TESTING THE ANTIBACTERIAL POWER OF BASIDIOMYCETES AND OTHER FUNGI DIFFICULT TO GROW IN LIQUID CULTURE
(Wilkins & Harris, 1944b)



The fungus to be tested was grown on a solid medium and when the colony was well developed radial strips were cut out of it and removed, together with the underlying agar. Warm agar was sown with a test organism such as a staphylococcus, and poured into a Petri dish. Before the agar set, the strips from the fungus colony were dropped into it. The plate was then incubated. The illustration shows inhibition by two species of Basidiomycete, *Tricholoma nudum* (1) and *Clitocybe aurantiaca* (2). In the former the older part of the fungus colony (c) has produced the most inhibitor, while in the latter the younger part (e) has produced the most.

similar way upwards of three thousand green plants have been examined by Osborn (1943; 1945; Fig. 13), and by others (for example, Cavallito, Buck & Suter, 1944; Cavallito, Bailey & Kirchner, 1945), with the discovery of many with an antibacterial action.

Heatley, in investigating (1944) the antibiotic produced by a small wild flower, *Crepis taraxacifolia*, elucidated an interesting phenomenon. He found that an inactive precursor was present in the plant and that the yellow petals of the flower contained an enzyme which, by acting on the precursor, produced the antibiotic. This he crystallized and

called crepin. As an antibacterial substance it is of little interest, although it has a powerful pharmacological action on the mammalian heart, but it is interesting to speculate in what way, if any, this enzyme-substrate reaction can benefit the plant, and whether its antibacterial powers have any significance.

From the surveys which have been made of scientific collections of fungi and of higher plants, as well as from the examination of stray contaminants, one sees that literally hundreds of producers of antibacterial substances are now known and that probably hundreds of new compounds await a comprehensive investigation.

Possible Further Uses of Antibiotics

Antibacterial action, however, is only one aspect of the potentialities of these substances, for their possible effects on protozoa and other non-bacterial causes of disease, particularly in the tropics, and on the fungi which cause skin infections, are as yet little explored. In this connexion there is interest in a substance which, it now appears, was first detected by Dutch workers (van Luijk, 1938) in the course of an investigation of *Penicillium expansum*. They eventually extracted a crystalline antibiotic which they called expansine, the same as that which has later been obtained from several other moulds and goes under a variety of names—clavacin, clavatin, claviformin, patulin, penicidin. This substance has a powerful antifungal effect and was used by the Dutch observers for treating fungus diseases of the skin. Sanders (1946) has found that many pathogenic fungi are inhibited by it at a dilution of at least 1 in 160,000, but it is toxic even when applied locally and its possible place in therapeutics remains to be seen. Both gramicidin and penicillin have been used in veterinary medicine, and the interest of antibiotics is not confined to human or animal therapeutics, for penicillin has been used successfully and apparently economically to treat the galls on apple trees caused by a susceptible bacterium, by injecting crude metabolism liquor containing penicillin into each gall (Brown & Boyle, 1944), and other bacteria and some of the fungi which cause plant diseases have been shown to be susceptible to antibiotics (Waksman & Bugie, 1943 ; Anslow, Raistrick & Smith, 1943 ; Gilliver, 1946).

Procedure of Antibiotic Research

The procedure for investigating substances which may be antibiotics is now fairly clearly established, and should be systematically followed. First the production of an antibacterial substance must be demonstrated. Usually moulds and bacteria are grown in liquid media and the liquid is examined by some such method as Heatley's cylinder-agar plate technique for inhibition of staphylococcus, *Bact. coli* or other selected organisms. This method of testing is equally suitable for testing any liquid extract and has been applied to the examination of plants and other possible natural sources.

Many basidiomycetes have been shown by Wilkins & Harris (1944a ; Wilkins, 1945) to produce antibacterial substances, and as most of these fungi grow very slowly in artificial culture and do not produce spores, the initial detection of their antibacterial action presents some difficulties. Wilkins and Harris grew the fungus on a solid medium and then with a sharp tool cut out a section passing through the centre of the colony. This section was embedded in an agar plate sown with bacteria, and if inhibitors were present they diffused out and inhibited the bacteria in the plate. It was possible by this means to see whether the younger or older part of the fungus produced the antibiotic (Wilkins & Harris, 1944b ; Fig. 14).

After it is demonstrated that an antibiotic is present, the first step towards the investigation of any substance is to find the best conditions for its production. In the case of fungi and bacteria it is a matter of finding the best cultural conditions and the best composition of the culture medium. With fungi in particular, the latter is often of the greatest importance, for a fungus may grow luxuriously and yet produce no antibiotic if the medium is not suitable, whereas on another medium, on which growth may be poorer, the antibiotic will be produced. With plants the production of the antibiotic has to be considered in relation to the season of the year, the phase of growth of the plant, the different parts of the plant, and so on. Then comes the examination of the best way to extract the substance. Though each extraction presents a new problem, experience has shown that certain lines of investigation should be followed. Many substances are soluble in organic solvents and most of these are relatively easy to purify, especially by means of chromatography. Neutral substances which are insoluble in organic solvents, however, still offer great difficulties in purification, but gradually methods are being evolved to deal with substances of this class.

After the substance has been extracted in a highly concentrated or pure form, the range of organisms against which it is active—usually pathogenic organisms are considered first—is determined, together with such data as whether it is inactivated by serum or body-fluids. The toxicity to whole animals, usually mice, is determined, and it is particularly important to determine the effect of repeated injections, for in chemotherapeutic practice repeated doses will always be necessary. For example, some antibiotics, of which a single injection is well tolerated, produce liver damage when the injection is repeated often. Toxicity to individual tissues is determined by *in vitro* observations on leucocytes or tissue cultures and by injections into the cerebrospinal cavity or other selected sites in animals. Pharmacological investigations into the effect of the substance on the most important physiological functions follow. Of these, the observations on the heart, blood pressure and respiration are the most important. If, at the end of these investigations, a substance appears to be both sufficiently powerful against bacteria and sufficiently non-toxic, protection experiments on appropriate animals, such as mice, or, for the tubercle bacillus, guinea-pigs, become justifiable. The infection must, of course, be induced by an organism sensitive *in vitro* to the antibiotic under test, and in order to determine the route and frequency of administration, and the size of the dose, studies on absorption and excretion in animals must be carried out.

The failure to carry out systematically this scheme of procedure undoubtedly accounts for the publication of many investigations which are far from complete. In any case, before serious trials on man are carried out, comprehensive laboratory work is necessary. One lesson has, I think, been clearly learnt of very recent years : that, in the field of antibiotic research, continuous and satisfactory progress can be expected only when people with different technical attainments and different outlooks agree to combine for comprehensive investigations. Few individuals have the knowledge and technical accomplishments to carry through all the necessary chemical and biological investigations completely.

The skilled investigation of the chemistry of such substances may lead to a better understanding of antibacterial action. The antibiotics so far known are mostly complex substances of large molecular weight, and the structure of few of them has so far been elucidated. As Dubos has insisted, much biochemical interest attaches to the clarification of the mechanism by which these substances produce their effects, for a thorough knowledge of this may bring with it the ability to construct, as it were, tailor-made chemotherapeutic agents suitable for every infection. From a still wider point of view the clear definition of these antibacterial substances may help us to understand the ceaseless struggle for existence which is being continually waged by microscopic organisms everywhere.

Fig. 1, 2, 3, 4, 6, 8 and 11 are reproduced from blocks kindly lent by the Editor of the *British Medical Journal*.

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ANALYSIS OF ANTIBACTERIAL ACTION

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Recovery of a host from infection implies that the conflicting interests of two species—host and parasite—have in the instance concerned been decided in favour of the host. We are at present interested in cases in which the recovery has been favoured by administration of substances to the already-infected host, and, in particular, in those cases in which the action of the chemotherapeutic agent concerned (or of a substance derived from it in the host) is upon the parasite. These instances include the majority of well-established chemotherapeutic actions, but it will be realized that the class of antibacterial agents is much more extensive than is the class of chemotherapeutic agents which affect bacteria. It includes many inhibitory substances whose behaviour in a host would make them unsuitable for chemotherapy. In order, however, to develop the study of antibacterial agents to a point at which it may be useful in chemotherapy, it will be advantageous not to emphasize the chemotherapeutic efficacy of the agents studied. The question of whether chemotherapeutic agents show any particular type of antibacterial activity can then be raised at a later point.

TYPES OF ANTIBACTERIAL ACTION

Growth of the parasite at the expense of the host is an essential part of infection and leads to the choice of growth and viability as criteria in classifying antibacterial effects into the following three types.

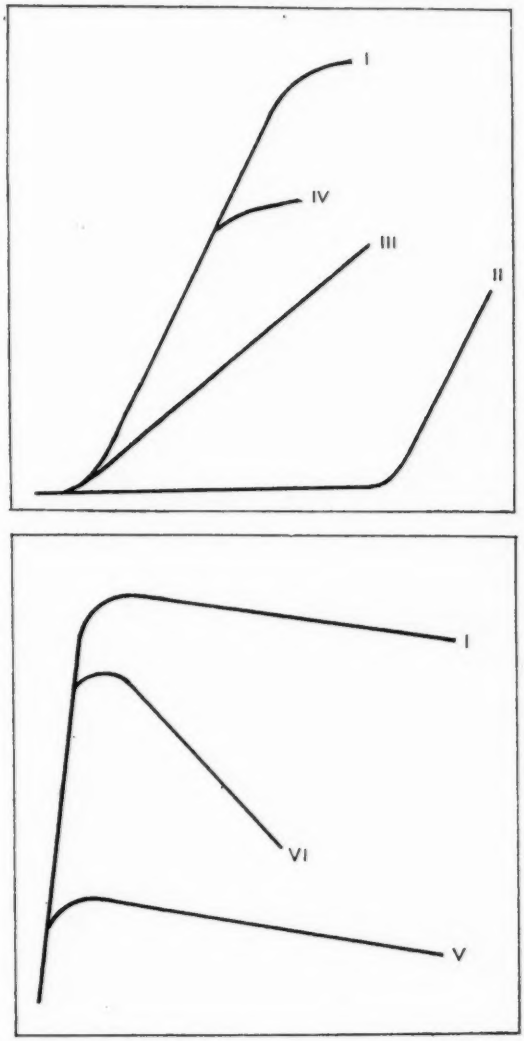
A. Actions on Bacterial Products, without much Effect on Growth and Viability

Such action becomes of interest to chemotherapy when the products affected are of types which are concerned in the relation between parasite and host; of these, the most important are antigenic substances, toxic to the host or protective to the organism. The use of antitoxins constitutes an important part of chemotherapy, but one which is conventionally studied apart from it. Of agents affecting protective substances, Dubos' findings (1939-40) are outstanding. By presenting a pneumococcal capsular polysaccharide as main nutrient to soil bacteria, a species was isolated which yielded an enzyme capable of breaking down specifically that polysaccharide, *in vitro* and *in vivo*. Growth of the pneumococci was otherwise normal *in vitro*, but *in vivo* the non-capsulated cells underwent phagocytosis and were unable to cause infection which proceeded in the absence of the enzyme. In this case the action of the agent was such as to afford an immediate experimental connexion with a specific constituent of the bacterial cell, of known function. This makes its action readily understandable: as will be seen below, much investigation is required before such knowledge of other types of agent is attainable.

B. Effects upon Growth

Effects on growth are many, and some description must precede attempts at their understanding. If initial observations are confined to the increase of bacterial substance during growth of cultures, ignoring the morphology of the cells so produced, the growth-curve becomes the basis of description. Curve i, Fig. 1, represents the course of growth commonly found in an uninhibited culture, and the effects which different agents have been observed to have upon such growth include the following. Increase in lag

FIG. 1 and 2. DIAGRAMS REPRESENTING THE COURSE OF GROWTH OF BACTERIAL CULTURES



Abscissae = time
Ordinates = logarithms of numbers of organisms
Fig. 2 covers a longer period of time than Fig. 1
Curve I in each represents normal growth; the other curves represent cultures under the influence of various substances, which are referred to in the text.

(as in curve ii) is the principal effect of sublethal concentrations of proflavine and methylene blue on *Bact. lactis aerogenes* (Davies, Hinshelwood & Pryce, 1944). Decrease in rate of growth (curve iii) is the first action produced on growth by sulphonamides (Kohn & Harris, 1941) and pantoyltaurine (McIlwain, 1944), and also one of the effects of concentrations of phenol, mercuric chloride, cupric salts and formaldehyde lower than those which are bactericidal (Poole & Hinshelwood, 1940). Limitation of stationary population (curve iv) is also caused by sublethal concentrations of phenol, $HgCl_2$, Cu^{++}, and formaldehyde, but the present effect can be shown to be secondary to the action on growth-rate (Poole & Hinshelwood, 1940). Adverse *p*H can lower stationary population with little effect on growth-rate.

C. Effects upon Viability

If investigation of the numbers of organisms in normal cultures is continued beyond their " stationary " population, as in Fig. 2, it is found that a slow death-rate ensues (curve i). A proportion of organisms is, in fact, dying even during the growth of the culture as a whole. This can become apparent during the action of a bacteriostatic compound, as in curve v. In addition, some compounds regarded as primarily bacteriostatic—such as the sulphonamides—may to some extent accelerate this process (Wolff & Julius, 1939 ; Colebrook & Cawston, 1945), but the agents termed bactericidal impose a much greater death-rate upon the culture, such as that represented by curve vi.

Until recently it did not appear that bactericidal agents were of great chemotherapeutic interest because their toxic action tended to show little specificity and to extend to animal tissues. Their potentiality has, however, been shown by penicillin, though the action of this substance is not one which has been regarded as typical of bactericidal agents. Phenol or formaldehyde can show differences in the intensity of their action upon bacteria at various stages of their growth, but with penicillin such differences become extreme. Penicillin had little action on staphylococci, streptococci or meningococci whose growth was prevented by lack of nutrients ; or, when nutrients were provided, by boric acid or by cooling. But under conditions which normally allowed bacterial growth, as in ordinary broth or serum, penicillin caused their death (Bigger, 1944 ; Hobby & Dawson, 1944 ; Miller & Foster, 1944 ; Hirsch, 1943-44). A further peculiarity of the action of penicillin on staphylococci appeared to be connected with its failure to act on non-proliferating organisms. This was the non-susceptibility to penicillin of a very small proportion of individuals in staphylococcal cultures. When, later, such organisms were caused to grow, they were of normal susceptibility to penicillin.

Bactericide by penicillin requires a definite period of exposure of organisms to the drug, and this period increases with decreasing concentrations of penicillin. Bactericidal effects initiated by penicillin could result in the death of organisms after their removal from penicillin-containing media (Parker & Marsh, 1946), an observation which suggests the existence of distinct stages, still to be defined, in its action.

Surveying the results of this initial classification of antibacterial action, one can observe characteristic differences in the effects of different compounds but little specificity to agents successful chemotherapeutically, and no adequate understanding of the origin of the actions. The effects have in many cases been ascribed to changes in hypothetical systems, and a physiological classification of such systems can be given in terms of their necessity for initiating or prolonging bacterial growth ; or in terms of their necessity (in systems affected by bactericidal agents) or at least partial dispensability (with bacteriostatic agents) for maintenance of bacteria. Further advance has been based on such initial analyses, but has employed different techniques. The necessity for correct initial recognition of the physiological characteristics of antibacterial action may be illustrated by the lack of advance in understanding of the action of sulphonamides, while they were believed to be primarily bactericidal ; or in that of penicillin, while it was believed to be primarily bacteriostatic.

BIOCHEMICAL AND CHEMICAL STUDIES

The main problem presented by the material of the foregoing paragraphs is the identification of the systems primarily affected by antibacterial agents. To approach this one may enquire to what extent growth is normally affected by chemical substances, apart from those artificially introduced. It is immediately found that many of the preceding effects can be paralleled by the action of substances normally associated with bacteria. Lag is increased by lowered concentrations of CO_2 (Gladstone, Fildes & Richardson, 1935 ; Dagley & Hinshelwood, 1938) or Mg^{++} (Lodge & Hinshelwood, 1939). Suboptimal concentrations of many substances essential to growth, including pantothenic acid (McIlwain, 1944), limit growth by a relatively specific action on the stationary population. Others affect growth-rate. It might therefore be possible to describe the systems attacked by inhibitors, in terms of the substances with which they normally interact.

Any investigation of the action of antibacterial agents meets at this point the complexity of living organisms, and the fact that more is unknown than is known of the nature and organization of their component systems. Empirical examination of the action of drugs on, for example, enzymes concerned with known nutrients would be extremely laborious and uncertain of yielding a positive result. Also, several inhibitory effects which have been discovered in this way have proved to be unconnected with the actions of the inhibitors on growth. Approaches which are more analytical and selective, and in some way specific to the particular agent concerned, are therefore required. Those which have been found successful do not conform to a single pattern, but are illustrated here by investigations of sulphonamide and acridine derivatives.

Sulphonamides and Drug-antagonists

Such an approach was found in the case of the *p*-aminobenzene sulphonamides, in the study of substances antagonizing their action. The inhibitory action of the drugs was found to be prevented by miscellaneous preparations of naturally-occurring materials, such as tissue or yeast extracts (Stamp, 1939), which did not, however, react with sulphanilamide in the absence of the bacteria. Supposing sulphanilamide action to have some degree of specificity, particular substances in the extracts might be expected to be associated with it. This proved to be the case and the main antagonist was shown to have the properties of *p*-aminobenzoic acid (Fildes, 1940 ; Woods, 1940). Here the selection of one compound related to the drug, from the many associated with living organisms, was achieved by chemical fractionation.

Degree of Action and Drug Concentration

The connexions frequently studied between such characters became of considerable value in the analysis of the action of sulphonamides, after *p*-aminobenzoic acid had been characterized. The discovery of this substance as antagonist was coupled with the finding of a rough proportionality between the concentrations of sulphanilamide and *p*-aminobenzoic acid needed to produce a given antibacterial effect, and the suggestion that this was due to competition between the two substances for essential bacterial systems. Such competition was expressed mathematically, assuming both compounds to be in equilibrium with such a system, and the rate of growth of *Escherichia coli* during its logarithmic phase was shown to be dependent on the quantity of *p*-aminobenzoate attached to the system (Wyss, 1941).

Assimilation and Function of *p*-Aminobenzoate

Some knowledge of this system has been obtained by considering the properties of *p*-aminobenzoic acid as it exists in bacteria. Organisms susceptible to sulphonamides may or may not synthesize *p*-aminobenzoic acid, but it exists in their cells in a combined form. If streptococci are grown in the presence of partly-inhibitory concentrations of sulphanilamide, they can suffer defects in major metabolic processes such as oxygen uptake (cf. Henry, 1943) ; but sulphanilamide cannot induce such deficiencies in already-grown cells. It does not displace from them a significant part of the *p*-aminobenzoic acid which they contain (McIlwain, 1945), but it does limit the cells in building up fresh cells containing *p*-aminobenzoic acid.

If sulphanilamide acts by inhibiting assimilation of *p*-aminobenzoic acid, the further question is raised of the manner in which this substance is essential to growth. Some suggestions regarding this have been made by a closer analysis of the course of growth of organisms in the presence of sulphonamides and of natural materials which antagonize their action. If complete growth-curves are followed, evidence for the occurrence of a type of antagonism different from that of *p*-aminobenzoic acid is found (Kohn & Harris, 1941 ; 1943 ; Harris & Kohn, 1941). This also can be ascribed to particular substances of natural occurrence, among which are methionine, certain purines, and further substances still unidentified. It is suggested that these compounds are the normal products of systems in which *p*-aminobenzoic acid functions, but the necessary biochemical demonstration of this is lacking.

For an approach to a full understanding of the action of sulphanilamide it will be necessary also to connect such findings with physiological features in the action of the inhibitors : are methionine and purines less necessary during reactions of the lag-phase in bacterial growth, on which sulphanilamide has less action, than during the logarithmic phase, which is more susceptible to inhibition ? The observation that methionine and purines are components of major cell-materials, such as proteins and nucleic acids, may be a partial answer, but detailed connexions between such observations and the defective respiration referred to above remain to be discovered.

Structural Specificity of the Inhibiting Agent

Sulphonamides have been discussed above collectively, but quantitative differences between the activities of differently-substituted *p*-aminobenzene sulphonamides have given evidence confirming that their main action is related to *p*-aminobenzoic acid. Investigation of the action of drugs, by examining a series of related compounds so that connexions can be found between their structure and action, is a well-established method, and one which follows naturally from that empiricism in chemotherapy which has demanded the production of many compounds before effective ones have been found. Connexions between structure and action in themselves give only limited information, but if a relationship is suspected between an antibacterial action and further characteristics in the action of a drug, then the degree to which the two features are correlated in a series of chemically-related compounds gives evidence on the validity of the suspected relationship.

The first characteristic in the sulphonamides to be connected with the differing abilities of the drugs to inhibit bacteria was the interacting ratios of the concentrations of the drugs and *p*-aminobenzoic acid (see above ; Rose & Fox, 1942). This confirmed the importance of *p*-aminobenzoic acid in their action. The second characteristic so correlated was the acidic-dissociation constant of the drugs. This property was selected for investigation on the basis of the finding that action of sulphonamides was related to *p*-aminobenzoic acid through competition for a common system of the bacteria ; it was considered that competition would be effective in so far as the sulphonamide approached *p*-aminobenzoate in its behaviour as an ionized amino-acid. Here a marked optimum in antibacterial activity at *p*H 7 was found to be associated with a *p*Ka of 6·5 (Bell & Roblin, 1942). This finding was of considerable value, as through the known effects of substituents on the ionization of aromatic derivatives, it enabled the probable action of unknown substances to be predicted.

Structural Specificity in the Action of Acridine Derivatives

This latter phase in the investigation of the action of sulphonamides has been paralleled in that of acridine derivatives. In this case, knowledge of the physical property likely to be concerned in their action was not obtained on the basis of prior biochemical investigation, but by studying several such properties in over 100 acridine derivatives (Albert, Rubbo, Goldacre, Davey & Stone, 1945). Several organisms of varying susceptibilities to the inhibitors were examined, but the relative activities of the compounds were roughly the same in each organism ; it was concluded that their actions in the different organisms were also similar. Among the different compounds, structures capable of resonance were frequently associated with high antibacterial activity, but not always ; lipophilic nature was not markedly correlated with antibacterial properties, and redox potential appeared of secondary and not primary importance. The closest correlation with antibacterial properties was found in the basic strengths of the compounds. The effect of substituents in the acridine molecule, upon antibacterial action in the resulting compounds, could largely be determined by their effect upon basic strength : amino-groups in the 2- and 5- positions increased both properties ; in chloro-derivatives, both were depressed unless the compounds substituted were already highly ionized ; hydroxyl-groups also depressed both properties. Nitro-groups were exceptional in introducing discrepancies into the above correlation, presumably through action of a different type from that typical of the acridine nucleus.

These findings were summarized in the conclusion that only those compounds of basicity sufficient for some 75% of the acridine to be present as cations at *p*H 7·3 were effective antibacterial agents. As the effects of substituents on the basic strength of aromatic molecules is known from independent data, in at least a semi-quantitative fashion, it was possible for the probable antibacterial potency of unknown molecules to be indicated and to afford a guide in synthesis.

Antibacterial action in the acridines fell with decrease of *p*H in a manner which was quantitatively interpreted as a competition between H ions and the bases, for essential sites on the bacteria. Growth or absence of growth was conditioned by the balance of the reactions (R being a bacterial grouping and A an acridine derivative): $R^- + H^+ \rightleftharpoons RH$ (capable of functioning) and $R^- + A^+ \rightleftharpoons RA$ (inactive). Little can be said of these groupings, from the present results, except that they are presumably acids with properties in some way related to the acridine nucleus; for not all bases act as do the acridines.

Antagonists to the Action of Acridine Derivatives

At this point some independent characterization of the bacterial system affected by acridine derivatives was required. Study of antagonists to the action of the compounds has not been as productive as was the case with sulphonamides, but has given some relevant data. Firstly, the naturally-occurring substances most potent in antagonizing proflavine (2 : 8-diaminoacridine) are nucleic acids, which act by forming with it complex salts (McIlwain, 1941). These are not necessarily precipitated, but reduce the concentration of proflavine ions in solution, superimposing on the equilibria of the preceding paragraph the further one (N^- = nucleic acid ion): $N^- + A^+ \rightleftharpoons NA$, through which some of the acridine derivative is withdrawn from the bacteria. Organisms exposed to proflavine until they were non-viable under ordinary conditions could be rendered viable by exposure to nucleic acids. Both the quantities of nucleic acid needed as antagonist, and features in the action of the acridines, suggest that their action is not to combine with all nucleic acids of the organisms, but with, for example, certain specific nucleic acids.

As with the suphonamides, different types of compound have been found to antagonize only limited concentrations of proflavine. In the case of *Escherichia coli*, one of these antagonists had the properties of an amino-acid but was not chemically identified (McIlwain, 1941). With *Bact. lactis aerogenes* such an antagonist was found to be of importance in the normal growth of the organism (Davies, Hinshelwood & Pryce, 1944). The main effect of proflavine on the bacterium was to increase the length of its lag-period. This could also occur independently of antibacterial agents; it was found in the growth of an inoculum taken from a very young culture, and was then annulled by addition of a sterile filtrate from an older culture. Such a filtrate would also prevent the increased lag due to proflavine. Substances of the filtrate were presumably normal products of bacterial enzymes, which may thus be concluded to be inhibited by proflavine. Here, also, the possibility is afforded of biochemical characterization of the system concerned. The filtrate did not antagonize all actions of proflavine; the cells remained morphologically distorted.

OBSERVATIONS ON THE INVESTIGATION OF ANTIBACTERIAL ACTION

From these and other examples, some general observations may be made with respect to current studies of antibacterial agents. It is apparent that the effect of any one agent is to be traced through many consecutive systems of the bacteria, and, indeed, study of such agents is making considerable contributions to understanding the organization of living systems in general.

Considering such systems and commencing with the initial point of action of the agent, it will be seen that the " receptors " of earlier investigators are now usually regarded as enzyme-systems. Probably not sufficient investigations have been carried out to show any limitations to this view, but it has been of great practical and theoretical value. In particular, it has enabled the gap between added substance and bacterial processes to be bridged by entities whose general properties are well known, although the isolation of a receptor concerned with antibacterial chemotherapeutic agents has not yet been achieved. Regarding drug-receptors as enzyme-systems has not only given a general understanding of the relationship between drug and bacterium, but has greatly assisted the quantitative study of their interaction. Structural relationship between certain antibacterial agents and compounds normally of importance to the organisms is not necessarily specific to this outlook, but investigation of such relationships has developed largely under the stimulus of enzyme theories.

Connexion between the products of the enzymes considered to be initially affected, and the physiological behaviour of the bacteria, is not by any means immediate. With sulphanilamide or pantoyltaurine the first action of the inhibitor appears to be on a system building up a further enzyme. The products of such an enzyme may be the substances of structural importance implicated as secondary antagonists to sulphonamides, or connected with energy-yielding reactions as in the case of pantoyltaurine; but it is apparent that many systems are involved whose isolation or further characterization is necessary before the actions can be said to be understood with any completeness.

In other instances—which include penicillin—investigations still require an initial directive comparable to that given to the study of sulphanilamide by the implication of *p*-aminobenzoic acid in its action. This stage would probably have been passed if the methods successful with sulphanilamide were directly applicable to penicillin. That element in the investigation of sulphonamides, pantoyltaurine, the antipneumococcal polysaccharidases and other agents, which is most probably of general application, is the great experimental value of establishing a connexion between their action and specific cell-constituents. Substance and process, here as elsewhere in living organisms, are interconnected aspects of the same entity, but, when neither is known, it is the substances that we can probably first characterize with greater certainty and precision. The development recounted above of knowledge of sulphonamide action illustrates how studies concentrating at one time on substances, and at another on processes, alternate and support each other. Study of antibacterial action is so far from being a stereotyped subject that the manner in which substances associated with new chemotherapeutic agents are characterized must depend on peculiarities of the agent concerned and on the genius of the individual investigator.

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THE NUTRITION OF BACTERIA

The Work of the Medical Research Council's Unit for Bacterial Chemistry

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Accurate knowledge of the nutrition of bacteria is of very recent date. At the time of the publication of the Medical Research Council's *System of bacteriology* (1929-31), it was understood that some bacteria of medical interest would grow on simple mixtures of salts in which the sole nitrogen source was NH_3, and lactate or glucose supplied the energy. On the other hand, it was known that other bacteria required amino-N. If these nutrients were inadequate, then " peptone " in its various forms was used and, in the last resort, serum, blood and other products of the " medium kitchen ". Those who were interested in the physiological aspects of nutrition—and they were few—had shown that an inadequate medium could be made adequate by the addition of extracts of organic material, and had even fractionated these extracts until the activity was so highly concentrated as to be capable of showing a biological effect in quantities as small as did an " accessory growth factor ". It was certainly supposed that the minute " accessory " addition was a single substance, and the influenza bacillus which was known to require two accessory factors was looked upon as more complex. Much discussion had taken place as to whether these were bacterial " vitamins ", comparable with the vitamins of animals.

Much the same position was taken up in the Medical Research Council's *Vitamins: a survey of present knowledge* (1932). The idea that bacteria required what may be called a balanced diet had not crystallized. Much work had been done on testing the reaction of bacteria to single substances as sole sources of carbon or nitrogen, but whatever interest such work may have, it had clearly no practical nutritional significance.

Ten years later the position had been greatly clarified as a result of work largely confined to America and Britain, in the latter by the Medical Research Council through its *Unit for Bacterial Chemistry*.

It became widely recognized that bacteria require for normal growth, not only the same basic sources of energy as do animals, but the same sources of amino-nitrogen and, within limits, the same vitamins. The only outstanding difference between animals and bacteria is due to the fact that the latter have more power of adaptation to changed environments, and can thus develop more easily a power to synthesize materials which are normally essential, and so dispense with these in the nutrients.

Preliminary Work on Bacterial Metabolism

The Unit (Department) for Bacterial Chemistry was founded by the Medical Research Council in 1934 in the following circumstances : The present writer, in the part-time service of the Council and working at the London Hospital, had been interested for years in the more physiological aspects of bacteriology, e.g. in the conditions of growth of the influenza bacillus (Fildes, 1920 ; 1921 ; 1922 ; 1923 ; 1924), and then in the conditions allowing the germination of tetanus spores (Fildes, 1927 ; 1929a ; 1929b ; Campbell & Fildes, 1931). Being without special chemical training, he became associated with B. C. J. G. Knight to study the germination of spores with more accuracy (Knight & Fildes, 1930). It then became clear that advance in this direction was unlikely without more knowledge of the basic nutrition of bacteria. At that time conditions were not unpropitious. Biochemistry was making increasing contributions to bacteriology. Dr. Marjory Stephenson, of Cambridge, had produced the first edition of her book, *Bacterial metabolism* (1930), in which knowledge of bacterial nutrition was critically analyzed from the biochemical point of view. In the first two volumes (1932, 1933) of *Annual Review of Biochemistry* she published articles on the chemistry of bacteria, in which nutritional advance was, however, so small as to be unworthy of mention. In the same volumes Heidelberger wrote on immuno-chemistry. It was only in the third volume (1934) that the nutrition of bacteria was referred to. Here it is recorded that Mueller, Klise, Porter & Graybiel (1933) in the USA had discovered that cystine and tryptophan were essential for the growth of *C. diphtheriae* ; that Burrows (1933), also in the USA, had

been growing *Cl. botulinum* on mixtures of amino-acids, and that Fildes & Knight (1933) in Britain had made several advances.

J. H. Mueller had been working on critical chemical lines for some years and, in fact, during his bacteriological studies, was the first to isolate a hitherto unknown substance, the amino-acid methionine, which later was shown to be an essential amino-acid for some microbes and, of course, for animals.

When starting work, Knight and Fildes decided to avoid non-pathogenic bacteria which, being able to grow on ammonium lactate, were clearly unsuitable test-objects for the study of "accessory factors". They concerned themselves with the more exacting, usually pathogenic, types which, in any case, were the microbes in which the Medical Research Council was directly interested. *Cl. tetani*, the germination of which had been under investigation, was, however, too "difficult", and work was transferred to *Cl. sporogenes*, which had similar anaerobic characters.

In due course they described a "Sporogenes vitamin" (Knight & Fildes, 1933), without which the organism would not grow. The chemical nature of this vitamin has never been established, in spite of the collaboration of Pappenheimer (1935), when he was on a visit to Britain and working at the Council's National Institute for Medical Research. In this respect the work of Knight and Fildes cannot be considered a major advance, but in the course of it they established to a large extent the amino-acid requirements of the organism. At that time, the impurity of natural amino-acids was a grave difficulty, and it was fortunate that W. C. Rose and his collaborators on animal nutrition laboured under the same disability in America, since this induced his colleague Marvel to evolve practical methods for the bulk synthesis of amino-acids. Gradually synthetic products are replacing the natural, but even to this day the series is not complete. The great importance of synthetic products for this type of work is shown by the fact that the growth of *Cl. sporogenes*, obtained in 1933 by Knight and Fildes, cannot be repeated at the present time. It is clear that some other essential factor, present as a contamination in the natural products then used, has been eliminated from our present reagents.

At this time Dr. G. P. Gladstone joined the writer and Knight as a bacteriologist, and papers (Fildes, Gladstone & Knight, 1933; Fildes & Knight, 1933) were published showing the amino-acid requirements of *Bact. typhosum* and recording the fact that the requirements changed on adaptation to a changed environment. The relation of tryptophan to nutrition was particularly stressed.

Thus an organism which normally required tryptophan could be trained to require it no longer, by gradually removing tryptophan from the nutrient mixture. This derived organism was found to have acquired the power to synthesize the missing tryptophan from simpler compounds. It was also pointed out that "the parasitic or pathogenic habit is apt to be related to inability to synthesize tryptophane".

Formation of the Medical Research Council's Unit

These papers indicated the possibility of a new approach to some aspects of medical bacteriology, and the Medical Research Council decided to form a special unit for continued research under the direction of the present writer, including Dr. Knight and Dr. Gladstone, and strengthening the biochemical side by the appointment of Dr. G. M. Richardson.

The Council was encouraged to take this step owing to the munificence of the Trustees of the late Viscount Leverhulme, who have maintained their support to the present day. The Council was also fortunate to receive a generous offer of accommodation for the Unit at the Bland Sutton Institute of the Middlesex Hospital, directed by Professor James McIntosh, an early colleague of the writer. This Unit was designed to be a partnership of bacteriologists and biochemists, working in adjacent rooms and in constant daily discussion. All papers published necessarily contained matter derived from or actually the work of anonymous colleagues.

Work was started at the Middlesex Hospital in 1934. The Unit was largely responsible for establishing for the first time the essential amino-acid requirements of a number of bacteria and for showing that nutritional requirements depended upon a failure of synthesis. Out of this work the concept was gradually developed of an evolution of bacteria depending on a loss of synthetic function, a concept precisely similar to that advanced simultaneously but independently by Lwoff at the Pasteur Institute, Paris, in relation to protozoa.

Whether the more speculative hypotheses on the evolutionary relations of animals and bacteria discussed by Fildes (1934) are rejected or not is of little importance, since they cannot be tested, but the data on which these were based—collected and correlated by Knight and published by the Medical Research Council (Knight, 1936)—created world-wide interest and, in fact, have been an important influence in stimulating the chemical study of bacterial nutrition.

The Unit became particularly involved in the nutrition of *Staph. aureus*. Richardson (1936) found that uracil was a necessary nutrient in the anaerobic growth of this organism. This was probably the first instance in which a bacterial vitamin, other than an amino-acid, had been precisely defined. Knight (1935) had been working on another new "factor" necessary for growth, and was later (1937) able to show that his factor consisted of aneurin and nicotinamide. Schopfer (1935) had already shown that aneurin was an essential nutrient for a mould, and Tatum, Wood & Peterson (1936) that it was probably essential for lactic bacilli. Nicotinamide was, however, a new introduction into bacteriology. These results made it clear that the use of the expression "vitamin" in relation to bacteria, rather than the more favoured "hormone", was correct, since one of these substances was already accepted as an animal vitamin while the other was accepted very soon after.

In the meantime Fildes & Richardson (1937) defined the sulphur requirements of staphylococcus, and Gladstone (1937) the nitrogen requirements and the conditions under which the haemolysin is produced (Gladstone, 1938). It was now possible for the first time to grow an exacting pathogen on a mixture of known composition.

At this stage Dr. G. M. Hills joined the Unit and established the necessity for aneurin in the pyruvate metabolism of *Staph. aureus* (Hills, 1938). Dr. Richardson left and was replaced by Dr. H. McIlwain, who became associated in the discovery of glutamine (McIlwain, Fildes, Gladstone & Knight, 1939) and of pantothenic acid (McIlwain, 1939) as factors in the growth of *Strept. haemolyticus*. In 1940 he was the first to grow *Strept. haemolyticus* on a defined mixture (McIlwain, 1940a).

In the meantime, a study of the nutrition of *B. anthracis*

revealed curious interrelationships between the nutrient amino-acids (Gladstone, 1939).

The Unit had repeatedly pointed out the close similarity between the amino-acid and vitamin requirements of animals and bacteria, and suggested that at least the preliminary stages of animal nutritional research could be established with bacteria with a great saving of time—in hours rather than in weeks or months. According to Williams (1943), this practice is now common. Microbiological tests "have the tremendous advantage that the number of tests can be very large without an inordinate expenditure of time".

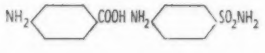
Influence of Bacterial Nutrition on Antiseptic and Chemotherapeutic Substances

Dr. Knight had now left the Unit and had been replaced by Dr. D. D. Woods, a member of the team directed by Dr. Marjory Stephenson working on bacterial metabolism for the Council at the Biochemical Laboratory, Cambridge. At that time Dr. L. E. H. (now Sir Lionel) Whitby, a member of the staff of the Bland Sutton Institute, was collaborating with Dr. A. J. Ewins on the testing of sulphonamide drugs, and informal discussions with the Unit were frequent. It seemed clear to the Unit that the action of an antibacterial drug might be by interference with the normal action of an "essential metabolite". The term "growth factor" had been abandoned in favour of this term, because the former was associated with nutrition, i.e. it was a substance which must be provided for a microbe which could not synthesize it. On the other hand, the same substance was an essential link in the metabolism of a microbe which could synthesize it, although it was not then required as a nutrient "growth factor".

The Unit had already shown that the group -SH was an essential metabolite, and since Hg is known to combine with -SH, it was not difficult to surmise that the antiseptic action of Hg might be due to this combination. It was also well known that the essential metabolite -SH (H-SH) could counteract or antagonize the antiseptic action of Hg. This hypothesis of the action of Hg was tested and found correct by Fildes (1940a).

In the case of sulphanilamide there was already evidence of the existence of "antagonizers" in extracts from bacteria (Stamp, 1939). These, in the view of the Unit, might well be essential metabolites on which sulphonamide reacted, but which in excess could antagonize sulphonamide.

This matter was taken up by Woods with the result that *p*-aminobenzoic acid was found to antagonize sulphonamide (Woods, 1940). According to Woods, *p*-aminobenzoic acid was an essential metabolite for bacteria, and the action of sulphonamide was due to interference with the normal metabolism of *p*-aminobenzoic acid, which, in turn, was due to the close structural similarity between these two substances :—



It was a question of competition between sulphanilamide and *p*-aminobenzoic acid for the service of an enzyme concerned normally with the metabolism of the latter.

A Rational Basis for the Development of Chemotherapeutic Agents

This was clearly a discovery of major importance, with many repercussions outlined by Fildes (1940b). Among other things, it indicated that competitive inhibitors might be designed to block the metabolism of any essential metabolite. It was only necessary so to modify the structure of an essential metabolite that it could no longer function as such, but still occupy the service of the enzyme. Thus, McIlwain (1940b) produced pyridine-3-sulphonic acid on the model of nicotinic acid and sulphonamide, and Fildes (1941) showed that indoleacrylate prevented growth by inhibiting the further utilization of indole, which substance he had found to be a stage in the synthesis of tryptophan (Fildes, 1940c). A rational procedure in the development of chemotherapeutic agents had thus been indicated, and at the same time a method for analyzing the stages of biosynthesis.

It was not given to the Unit to support the hypothesis by demonstrating the correctness of the prediction that *p*-aminobenzoic acid was a hitherto-unknown essential metabolite and animal vitamin, because it ceased work in the middle of 1940. Rubbo & Gillespie (1940) in Australia proved the first prediction and Ansbacher (1941) the second. Of the Unit, only Dr. McIlwain, at the University of Sheffield, continued this work.

Continuation of the Work in the United States during the War

During the war years the members of the Unit have watched the development of this subject in America with interest. The discovery of new bacterial vitamins has become almost a matter of routine, and the application of these discoveries to animal nutrition has become general. Van Niel (1943) states : "It is almost a foregone conclusion that a newly discovered growth factor, needed in minute amounts by some micro-organisms, will soon be shown to be a vitamin for higher organisms."

It would seem that the general hypothesis of the Unit, relating antibiotic action to the structure of essential metabolites, has been supported by many new instances and generally accepted. Thus, Daniels (1943) : "From the standpoint of biochemistry, the most interesting development in this class of compounds is the elucidation of a mechanism that allows one to correlate the activities of these compounds with their physico-chemical properties. Earlier postulates relating to the mechanism of action have been more or less superseded by the theory of Woods and Fildes."

Van Niel (1943) : "It must be recognized that the attempts to produce new bacteriostatic agents as analogues of known growth factors have resulted in products whose activity is, on the whole, so well in agreement with expectation that the fruitfulness of the original concept has been established."

Williams (1943) : "While it must be admitted that the new approach to chemotherapy has not, as yet, led to the discovery of new therapeutic agents of outstanding merit, it seems likely that a more thorough study of the fundamental facts concerned will bear fruit."

In view of these opinions, too much attention need not be paid to the few objectors. There has been a tendency to generalize far beyond any claims made by Woods in his only contribution to this subject (1940).

The Unit had just embarked upon the analysis of the stages in the biosynthesis of essential metabolites, using bacteria which were, spontaneously or by training, devoid of certain synthetic enzymes. This subject has now made great progress in America through a new technique of employing the mould *Neurospora*, in which single synthetic enzymes have been destroyed by the action of *x* rays. The

importance of this work rests on the fact that any stage in the synthesis of an essential metabolite is itself an essential metabolite and, further, one against which a competitive inhibitor can be designed.

Present State of Knowledge of Bacterial Nutrition

At the present time the general background of bacterial nutrition has been established, although there are lacunae to be filled. One can say that nutrients are required in the last resort to allow bacteria to reproduce and, of course, to carry on functions of which the appointed end is reproduction. These activities require free energy and the basic materials from which new protoplasm, of the type specific to the bacterium, can be built.

If the argument is confined to the more or less heterophilic bacteria which include those of medical interest, we can say that the science of nutrition defines the precise chemical ingredients which must be absorbed by a bacterium so that (*a*) it can liberate free energy from them by katabolic enzymes and (*b*) build up protein from them by synthetic enzymes.

In order that the bacterium may absorb these ingredients they must be of a molecular size small enough to allow diffusion into it.

The mechanisms by which energy is liberated have been studied for years under the heading of " bacterial metabolism ", and the Unit has had little concern with this aspect of the subject. Its chief interest has been in the synthetic processes.

Here we have nitrogen-containing substances of small molecular weight, even as small as ammonium salts, and inorganic substances containing the other essential elements. These are absorbed by bacteria and in a few hours synthesized to protein. This remarkable feat is, of course, not achieved in one step, but in a series of steps, eacn achieved by a separate synthetic enzyme.

Some bacteria have a full battery of these enzymes, so that they can proceed from NH_3 to protein unassisted. Other bacteria have not, and in this case cannot grow because the synthesis is blocked at the level of the enzyme deficiency. It is these bacteria that throw light on nutrition, because they can grow only when the missing component is supplied to them preformed as a nutrient. This is the " growth factor " singled out by a particular bacterium, from all the other " essential metabolites " in the synthetic chain, by the fact that it cannot synthesize it.

Plans for Resumption of the Unit's Work

The Medical Research Council is now concerned with reconstructing the Unit to continue this work and develop it along such lines as may be possible. Necessarily, there must be a long period of delay before results can flow. With the lapse of time, normal dispersal of personnel has taken place, Dr. G. P. Gladstone being the only survivor of the original Unit to assist in reconstruction. Dr. Woods is now organizing a new group at the University of Oxford. Dr. McIlwain remains at the University of Sheffield, and Dr. Hills is stationed at the Microbiological Research Department, Porton. Their places have been taken by Dr. H. N. Rydon, Dr. M. R. Pollock and Dr. D. Herbert. The former premises at the Middlesex Hospital, although little affected by the War, are now too small, and arrangements have been made to house the Unit at the Lister Institute of Preventive Medicine, where work will gradually develop as the present austere conditions improve.

The object of the Council in reconstructing the Unit may be supposed to be the discovery of fundamental knowledge of the physiological reactions of bacteria. Normally, work of this sort would be the concern of universities rather than of a Government Department charged with research for the alleviation of the sick, but in Britain bacteriology has always been so bound up with medicine that the universities have made little provision for it. The Council has long recognized that the application of research to the needs of man requires capital, and that this capital is the knowledge collected by those who work without any obligation to achieve utilitarian ends. They have thus taken over the duty of universities, or have at least by their example and assistance to universities started a movement which will provide material suitable for exploitation.

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MICROBIAL RESISTANCE TO CHEMOTHERAPEUTIC DRUGS

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Micro-organisms vary widely in their natural susceptibility to chemotherapeutic drugs, and this is particularly true of different species of bacteria, as is well shown by the relative insensitivity of the gram-negative bacilli to many of these agents. Natural variations in sensitivity are also common within the same species; thus, of 157 strains of *Staphylococcus aureus* isolated from clinical infections 9% were at least 8 times more resistant to penicillin than the average staphylococcal strain (Selbie, Simon & McIntosh, 1945). Such differences in natural susceptibility, not only to penicillin but also to the sulphonamides and other drugs, are encountered in many types of pathogenic micro-organisms and present a problem of considerable importance in chemotherapy. Naturally-occurring drug-resistant strains of micro-organisms, however, are usually unsuitable for investigation because their past history is usually unknown, and our knowledge of drug-resistance has therefore been derived mainly from the study of micro-organisms that have become resistant during the course of test-tube experiments or during the treatment of experimental or clinical infections.

The experimental method used in developing resistance to drugs and other toxic substances has in general consisted in exposing the micro-organism to increasing concentrations of the substance in serial subcultures, or by passage in animals which are treated in successive passages with increasing doses of a chemotherapeutic agent. The first experiments of this kind were done by Ehrlich and his colleagues in their studies on the development of resistance to dyes and arsenical drugs in trypanosomes, and in recent years this method has been extensively used in the study of bacterial resistance to the sulphonamides and penicillin. This method, however, has also been used with many other toxic substances, and Table I shows some of the many drug-resistant micro-organisms that have been developed in this way. Yeast has also been made resistant to pyrithiamine (Woolley & White, 1943), and tissue-cultures of metazoan cells have acquired increased tolerance to copper sulphate and sodium arsenite (Wilson, 1922) and to sulphonamides (Jacoby, Medawar & Willmer, 1941).

TABLE I. EXAMPLES OF MICROBIAL RESISTANCE TO DRUGS

Drug	Organism	Reference
Optoquine	Pneumococcus	Morgenroth & Kaufmann, 1912; Lewy, 1925
Lysozyme	*M. lysodeikticus*	Fleming & Allison, 1927
Acriflavine, Methyl violet	Staphylococcus	Burke, Ulrich & Hendrie, 1928
Proflavine, Propamidine, Quindoline	Staphylococcus	McIntosh & Selbie, 1943
Gramicidin	Staphylococcus	Philips & Barnes, 1942
Tyrothricin	Staphylococcus	Rammelkamp, 1942
Streptomycin	Gonococcus	Miller & Bohnhoff, 1946
Streptomycin	Meningococcus	Miller & Bohnhoff, 1946
Arsenic	*Sp. recurrentis*	Margulies, 1910; Gonder, 1912b; Fischl & Singer, 1934
Arsenic	*Sp. gallinarum*	Margulies, 1910; Gonder, 1912b
Gold	*Sp. recurrentis*	Fischl & Singer, 1934

It would thus appear that the ability to acquire tolerance to toxic substances is a common biological property of living cells and that the mechanism underlying the development of resistance is probably unspecific, particularly in view of the multiplicity of agents to which resistance can be acquired.

Drug-resistance in Trypanosomes

The development of drug-resistance was first observed in mice infected with *Trypanosoma brucei* by Browning, Franke and Roehl (Ehrlich, 1907; Browning, 1907), who found that the trypanosomes which appeared in the blood in relapses after treatment with parafuchsin required more and more of the dye to ensure disappearance after successive relapses, until safe doses of the dye were no longer effective. It was soon found that in trypanosomes resistance could be developed to other toxic substances, and that resistance could be increased by transferring the resistant relapse strains to other mice which were then treated with increased doses of the appropriate drug. Trypanosomes made resistant to drugs in this way were later shown by *in vitro* tests to survive higher concentrations of the appropriate drugs than the parent sensitive strains (Mesnil & Brimont, 1908; Ehrlich, 1909a; Neven, 1909) and could thus be differentiated from trypanosomes that were resistant to treatment by having become tolerant to specific antibodies, a type of acquired resistance that was demonstrated in trypanosomes by Ehrlich (1909b) and Levaditi & McIntosh (1910).

Drug-resistance in trypanosomes can also be demonstrated by their reduced affinity to dyes and other compounds. Trypanosomes resistant to a particular dye remain colourless when placed in solutions of the dye and allied compounds, whereas the normal parent strain of trypanosomes becomes deeply stained. Similarly, arsenic-resistant trypanosomes have been shown by Levaditi (1909) in collaboration with McIntosh and by Yorke, Murgatroyd & Hawking (1931) to fix less arsenic than normal trypanosomes. Resistance to aromatic arsenical drugs in trypanosomes is also accompanied by resistance and diminished affinity to acridine, oxazine and other dyes (Ehrlich, 1909b; Gonder, 1912a), so that it would appear that resistance is directed not against arsenic but against the side-chains in the phenyl radical. Drug-resistance in trypanosomes would therefore seem to be due to a failure in the primary fixation of the drug, and not to interference with the final toxic action of the drug on some essential substrate.

Another change that has been noted in trypanosomes during the development of resistance to acridine and oxazine dyes is a disappearance of the parabasal body or blepharo-

plast (Werbitzki, 1910; Kudicke, 1911; Laveran & Roudsky, 1911). There has been considerable discussion on the meaning of this structural change, but it would certainly appear to signify that there has been a variation or mutation during the development of resistance. Evidence that resistance can arise from natural variation is also afforded by the sudden appearance of arsenic-resistance in trypanosomes during passage in normal animals (Morgenroth, 1924; Browning, 1931). A recent and well-documented report by Eagle & Magnuson (1944) on a strain of trypanosomes that spontaneously acquired arsenic-resistance is of particular interest, because this strain displayed the same characters of reduced affinity to drugs and chemical specificity as are shown by experimental arsenic-resistant strains. It would therefore appear probable that the mechanism underlying the development of drug-resistance in trypanosomes is that of a progressive selection of spontaneous variants, the drug allowing the survival of only the more resistant variants. Further discussion on this problem and on the question of the specificity of drug-resistance is to be found in the communications of Browning (1931), King & Strangeways (1942) and Eagle & Magnuson (1944).

Resistance to Sulphonamides

The development of resistance to sulphonamides was first reported by Maclean, Rogers & Fleming (1939), who found an increase of resistance in pneumococci from patients undergoing treatment with sulphapyridine. Since then there have been numerous reports of increased resistance to sulphonamides during the treatment of clinical infections and, but for the advent of penicillin, sulphonamide-resistance in gonorrhoea would have become a problem of grave epidemiological importance. Among the first studies on the development of resistance to sulphonamides by experimental methods were those of MacLeod & Daddi (1939) on pneumococci, Chandler & Janeway (1939) on streptococci and Green (1940) on *Br. abortus*. Sulphonamide-resistance has since then been developed experimentally in many other types of bacteria, including staphylococci, *Neisseriae*, *Corynebacteria*, *Haemophili*, organisms of the coli-typhoid group, and even in one of the larger viruses, that of lymphogranuloma venereum (Jones, Rake & Stearns, 1945). Sulphonamide-resistance is in general agreed to be irreversible, whether it is natural or acquired.

MacLeod (1939) has reported that the *in vitro* development of resistance in pneumococci is associated with a loss of the power to dehydrogenate glycerol, lactate and pyruvate and with the production of less hydrogen peroxide. Kohn & Harris (1942) have observed that sulphonamide-resistant bacteria are more exacting in their nutritional requirements, while Sevag & Green (1944) have associated sulphonamide-resistance with an alteration in glucose metabolism. On the other hand, Wyss, Strandskov & Schmelkes (1942) have shown that the *in vitro* development of sulphanilamide-resistance in *Bact. coli* did not reduce the susceptibility of respiration in the resting cells to inhibition by sulphanilamide.

Of greater interest, however, is the finding that in some bacteria the development of resistance is accompanied by increased production of substances that inhibit the antibacterial action of the sulphonamides. This was first observed by the American worker, MacLeod (1940), in pneumococci that had acquired resistance to sulphapyridine *in vitro*, and it was confirmed by Tillett, Cambier & Harris (1943) with MacLeod's cultures, but not with two sulphonamide-resistant strains of pneumococci from clinical cases. A similar finding was made in sulphanilamide-resistant *Br. abortus* by Green (1940), and Green & Bielschowsky (1942) were able to identify part of the sulphanilamide-inhibitor produced by resistant *Br. abortus* with *p*-aminobenzoic acid, following Woods' (1940) demonstration of the anti-sulphonamide properties of this substance and his suggestion that sulphonamide-resistance in bacteria might be due to increased production of *p*-aminobenzoic acid.

Further evidence of this mechanism of resistance in staphylococci has been provided by Landy, Larkum, Oswald & Streightoff (1943) who, using a microbiological test for *p*-aminobenzoic acid with *Acetobacter suboxydans* (which requires this substance for normal growth), have found that staphylococci which have developed resistance to sulphathiazole produce more *p*-aminobenzoic acid than the parent-sensitive cultures. This property of sulphonamide-resistant staphylococci has been confirmed by Spink, Wright, Vivino & Skeggs (1944), and by Housewright & Koser (1944), who find that the production of *p*-aminobenzoic acid increases *pari passu* with the development of sulphonamide-resistance.

Similar findings have been made in cultures of gonococci rendered resistant to sulphonamide by Stokinger & Charles (1942) who, however, are of the opinion that the increased production of *p*-aminobenzoic acid does not account for the sulphonamide-resistance because the addition of azochloramide, which inhibits *p*-aminobenzoic acid, fails to overcome the resistance of the gonococcal cultures to sulphonamides. Landy & Gerstung (1944) have also measured the synthesis of *p*-aminobenzoic acid by gonococci isolated from 175 patients subsequently treated with sulphathiazole, and have found that in general greater amounts are synthesized by strains from patients that are resistant to treatment.

Increased production of *p*-aminobenzoic acid is, however, a far from constant finding in sulphonamide-resistant bacteria. Among the findings negative in this respect are those of McIntosh & Selbie (1943) with streptococci, Tillett, Cambier & Harris (1943) with pneumococci, Landy, Larkum, Oswald & Streightoff (1943) with *Bact. coli*, *Vibrio cholerae*, *Sh. dysenteriae* and pneumococci, and Housewright & Koser (1944) with *Sh. paradysenteriae* and pneumococci. It would thus appear that it is only in some bacteria that sulphonamide-resistance can be ascribed to an increased production of *p*-aminobenzoic acid, which counteracts the interference of sulphonamides with the metabolism of this substance (Woods, 1940). In other bacteria, it is possible that another type of sulphonamide-inhibitor is produced which cannot at present be detected, or that their metabolism is so altered that the use of *p*-aminobenzoic acid can be dispensed with.

A good example of different mechanisms being involved in the development of resistance has been given by McIlwain (1943). He finds that resistance to the toxic substance pantoyltaurine in *C. diphtheriae* depends on the ability of this organism to synthesize pantothenate, which is a specific inhibitor of pantoyltaurine in much the same way as *p*-aminobenzoic acid is an inhibitor of sulphanilamide, whereas pantoyltaurine-resistant streptococci differ from their parent-sensitive strains in possessing metabolic processes which are alternative to those involving pantothenate and are susceptible to inhibition by salicylate. The mechanism of sulphonamide-resistance in some bacteria may also prove to be of a less specific nature, such as lessened permeability, and thus be in a sense comparable to the mechanism of drug-resistance in trypanosomes, where resistance is directed

against the access of the drug rather than against its specific toxic action on the sensitive substrate.

There have been conflicting reports on the effect of the development of sulphonamide-resistance on the virulence of bacteria to experimental animals. MacLeod & Daddi (1939) have found no change in virulence in pneumococci or streptococci rendered resistant to a sulphonamide by the *in vitro* method. Horsfall (1942), however, has observed a considerable reduction in virulence in pneumococci after the *in vitro* development of resistance to sulphathiazole. This has also been the experience of Schmidt, Sesler & Dettwiler (1942) in sulphonamide-resistance experiments with pneumococci, using the *in vitro* method, but the same strains of pneumococci have shown no appreciable loss of virulence after the induction of resistance by passage through treated mice. Spink, Hall & Ferris (1945) also find that staphylococci retain their virulence after having acquired resistance to sulphonamides by both the *in vitro* and *in vivo* methods. The available evidence thus indicates that the *in vitro* method of developing resistance may lead to a loss of virulence, whereas the *in vivo* method has apparently little or no effect.

Penicillin-resistance

Acquired resistance to penicillin was first observed by Abraham, Chain, Fletcher, Florey, Gardner, Heatley & Jennings (1941), who induced penicillin-resistance in staphylococci by the *in vitro* method. Other workers have induced penicillin-resistance by *in vitro* and *in vivo* methods in staphylococci, and also in pneumococci, streptococci, meningococci and gonococci. Increased bacterial resistance to penicillin has also been observed during the treatment of clinical cases with penicillin, especially in staphylococcal infections, but the increases in resistance that have so far been encountered do not appear to have interfered with the efficacy of treatment, provided that the dosage of penicillin is adequate (Selbie, Simon & McIntosh, 1945). Penicillin-resistance that has been developed by cultural methods has proved reversible in the hands of Todd, Turner & Drew (1945) with staphylococci and streptococci, but not pneumococci, and this finding has been confirmed in the case of staphylococci by Spink, Hall & Ferris (1945). Loss of resistance to penicillin, however, has not so far been observed in bacteria that are naturally resistant or have acquired resistance in patients or animals undergoing treatment with penicillin.

Some of the modifications that have been found in association with the development of resistance to penicillin are an increase in size of staphylococci (Smith & Hay, 1942) and a reduction in growth-rate and enzymic activity in staphylococci (Abraham *et al.*, 1941) and in pneumococci (McKee & Houck, 1943). Of greater importance, however, is the evidence that resistance may be related to the production of penicillinase, the penicillin-destroying enzyme first isolated from gram-negative bacilli by Abraham & Chain (1940). Rake, McKee, Hamre & Houck (1944) were unable to detect the production of penicillinase by staphylococci that had been made resistant to penicillin, and this has also been the experience of Spink, Hall & Ferris (1945) and Bondi & Dietz (1946). In staphylococci isolated from clinical infections, however, Kirby (1944), Bondi & Dietz (1945) and Gots (1945) have shown that penicillin-resistant strains almost invariably produce considerable amounts of penicillinase. That resistance in many of these strains of staphylococci is due to the production of penicillinase has been demonstrated by Luria (1946), who finds that, when tested *in vitro* with small inocula, they are fully sensitive to penicillin, their growth being inhibited before sufficient penicillinase is produced to destroy the penicillin. There are organisms, however, which are normally highly resistant to penicillin and are apparently unable to produce penicillinase, such as *Bact. typhosum* and certain organisms of the salmonella group (Bondi & Dietz, 1944). It would thus appear that, although the production of penicillinase may account for the natural resistance to penicillin of some organisms, it does not necessarily follow that the inability to produce penicillinase implies sensitivity, or that the development of resistance by experimental methods is associated with the increased production of penicillinase.

The effect of the development of resistance to penicillin on the virulence of bacteria seems to depend largely on the method used for inducing resistance, as has already been mentioned regarding sulphonamide-resistance. McKee & Houck (1943) have found that streptococci and pneumococci are greatly reduced in virulence to mice after being made resistant to penicillin by the cultural method and that virulence cannot be restored by passage through mice. In similar experiments with 7 strains of meningococci, Miller & Bohnhoff (1945) have also observed considerable and irreversible falls in virulence to mice during the *in vitro* development of resistance. On the other hand, there has been no decrease in virulence to mice in pneumococci or staphylococci that have been made resistant to penicillin by passage through treated mice (Schmidt & Sesler, 1943; Rake, McKee, Hamre & Houck, 1944).

This difference in the effect on virulence between the *in vitro* and *in vivo* methods of inducing resistance to penicillin and the sulphonamides is to be expected when consideration is taken of the environmental conditions. The virulence of bacteria tends to fall with repeated sub-culture, particularly when the medium is unfavourable as is the case here, where sub-effective concentrations of a bacteriostatic agent are used for the induction of resistance. On the other hand, the passage of bacteria through animals tends to maintain or increase virulence so that, in the *in vivo* method, resistance is developed only in organisms that are adapted to survival in the host tissues. The influence of this factor is well shown in the experiments of Rake, McKee, Hamre & Houck (1944), where a strain of staphylococci, rendered resistant to treatment by passage through penicillin-treated mice, was, however, fully sensitive to the action of penicillin *in vitro*, and must therefore have been resistant to treatment by having adapted itself to the antibacterial action of the tissues of the mouse. In this respect the situation regarding the *in vivo* development of resistance in bacteria to treatment with chemotherapeutic agents is similar to that afforded by the development of serum-resistance in trypanosomes which, as has already been mentioned, has long been recognized as a complicating factor in the assessment of drug-resistance (Browning, 1931).

Cross-resistance

It has been stated that, when an organism is resistant to one sulphonamide, it is correspondingly resistant to all other sulphonamides. It is now recognized that, although there may be some cross-resistance within the sulphonamide group, it is not quantitatively complete (Kirby & Rantz, 1943), and Colebrook (1943) has shown that, of 7 strains of streptococci resistant to sulphonamide, only one was resistant to three

other sulphonamides and six were fully sensitive to sulphathiazole. This lack of complete cross-resistance within the sulphonamides is to be expected if one assumes, as has been suggested here, that the mechanism of sulphonamide-resistance may vary from one organism to another.

It has been agreed by many workers that an organism that has acquired resistance to one drug, such as penicillin or one of the sulphonamides, still retains its sensitivity to other unrelated drugs. McIntosh & Selbie (1943), however, showed that staphylococci made resistant to proflavine (2:8-diaminoacridine), or to the unrelated drug propamidine, were resistant to both compounds, while proflavine-resistant cultures had acquired no increased resistance to the closely related compounds 2:7-diaminoacridine and 5-aminoacridine. The two latter compounds also differed from proflavine, in that resistance to them could not be developed in staphylococci. Albert, Rubbo, Goldacre, Davey & Stone (1945) have suggested that these differences may be only a measure of the facility of the resistant organisms to destroy a drug by oxidation or deamination, and that it would therefore be impossible to develop resistance to such a highly stable substance as 5-aminoacridine. In any case, it is apparent from the close relationship between resistance to proflavine and propamidine that resistance is directed against the side-chains in much the same way as has already been mentioned regarding the resistance to dyes of arsenic-resistant trypanosomes. McIntosh & Selbie also found that two cultures of staphylococci that were made resistant to quindoline methochloride differed, in that one was also highly resistant to propamidine whereas the second showed no such cross-resistance. This finding again shows that organisms may develop resistance to the same drug in different ways.

Mechanism of Development of Resistance

The process involved in the development of drug-resistance does not appear to be one of selection from resistant organisms already present in the parent culture, because the organisms in the final resistant culture are more resistant to the drug than any single member of the original culture (McIntosh & Selbie, 1943). This process must therefore involve an adaptation of the organism to the presence of the drug so that it becomes less sensitive. The adaptation of the organism, however, does not appear to be a specific response to the drug, because organisms made resistant to the same drug can differ in their mechanism of resistance and in other properties. Such differences are to be found in the production of *p*-aminobenzoic acid or penicillinase in sulphonamide- or penicillin-resistant bacteria, and in the effects of the development of resistance on virulence.

The mechanism underlying the development of resistance would therefore appear to be of an unspecific nature, and is probably an aspect of the capacity for spontaneous variation or mutation which is a property common to all micro-organisms. Evidence in support of this view has been adduced by Demerec (1945), who finds that the development of resistance to penicillin in staphylococci is proportional to the mutation-rate. Further supporting evidence is given by Carpenter, Bahn, Ackerman & Stokinger (1945) in their *in vitro* experiments on the induction of resistance in 7 strains of gonococci with single drugs and mixtures of drugs. When single drugs were used, the average increases of resistance that were obtained with sulphathiazole, rivanol lactate, promin and penicillin were 700-, 80-, 9- and 182-fold respectively, but when resistance was induced in a mixture of the first three drugs, there was only an 8-fold increase in resistance to each of the three drugs, and when all four were used there was no increase of resistance to any of the four drugs. On the other hand, resistance to more than one drug can be elicited in an organism with ease, provided that the drugs are presented successively (McIntosh & Selbie, 1943).

These results are consistent with the view that the development of resistance depends ultimately on spontaneous variation, because the chance of the appearance of a successful variant would be progressively lessened as the number of factors required simultaneously for success was increased, the requisite factors here being resistance to different drugs. The role of the drug in the development of resistance would then be to eliminate the more sensitive organisms, and allow the survival of only the more resistant variants, and would thus be in a sense similar to the part played by lactose in providing a preferential medium for the encouragement of the growth of the spontaneous lactose-fermenting variants of *Bact. coli-mutabile* (Lewis, 1934). The process of the development of drug-resistance could thus be described as a progressive selection of spontaneous variants which have increased resistance to the drug.

Resistance in Clinical Infections

It is remarkable that, in spite of the widespread use of the sulphonamides and penicillin, there are relatively few instances in which organisms have been proved to have developed drug-resistance during the treatment of clinical infections. Furthermore, the development of resistance has been observed mainly in pneumococcal and staphylococcal infections, and rarely, if ever, in streptococcal infections. It would thus appear that some organisms are more prone than others to develop resistance in clinical infections, but it may be that the determining factor is the nature of the lesion. Thus, the relative avascularity of staphylococcal lesions would prevent the access of a bacteriostatic concentration of the drug to the infecting organism, and would thus provide the conditions necessary for the development of resistance (Hudson, Meanock, McIntosh & Selbie, 1946). In the case of streptomycin, however, the development of bacterial resistance occurs readily during the treatment of clinical infections (Buggs, Bronstein, Hirshfeld & Pilling, 1946), but this chemotherapeutic agent is exceptional, in that cultures of bacteria can acquire resistance to its action with extreme rapidity (Miller & Bohnhoff, 1946).

Increased resistance to treatment in clinical infections may be due to factors other than the development of resistance in the infecting organism. The original susceptible organism may be eliminated and replaced by a less susceptible type. This has been found by Siegel & Karr (1945) to occur during the prophylactic use of sulphadiazine in an epidemic of pneumococcal infections in children, and in the suppression of throat streptococci in naval training schools by Damrosch (1946).

The remarkable increase in the resistance of gonococcal infections to sulphonamide treatment may also be due to the gradual elimination of the more sensitive strains, so that only the more resistant strains are propagated. In support of this view is the American observation that the anticipated war-time rise in gonorrhoea has not occurred (Mahoney & Van Slyke, 1945), so that it must be presumed that the

sources of infection have been greatly reduced by sulphonamide therapy. It has also been observed by Harkness (1944) and Mahoney & Van Slyke (1945) that gonococcal infections may be highly resistant to sulphonamide treatment, although the infecting organism is fully sensitive to the drug *in vitro*, and it has been found that the serum of some of these cases contains a substance that counteracts the action of sulphonamides *in vitro* (Harkness, 1943).

It is thus evident that in the assessment of the development of resistance to treatment in clinical infections there are other factors to be considered before it can be concluded that the infecting organism has acquired drug-resistance.

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CHEMOTHERAPY OF RICKETTSIAL AND VIRUS DISEASES

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The essential nature of the smaller viruses is much disputed : the larger ones are generally agreed to be closely related to bacteria. They behave as obligate intracellular parasites of animals, higher plants or bacteria, and have not yet been cultivated outside the living cells of their host. They seem to have virtually no active metabolism when outside their host cell : what happens inside is hard to determine. Though smaller than most bacteria, viruses cannot be defined solely on a basis of size or filterability, for the largest are larger than the smallest bacteria. Between the main body of the viruses and the bacteria lie two fairly distinct groups, the rickettsiae and the viruses of the psittacosis group (Andrewes, 1944). The rickettsiae are conventionally excluded from the true viruses ; they are arthropod-transmitted, often rod-shaped, and show, many of them, antigenic cross-reactions with proteus bacilli ; but, like the viruses, they are intracellular parasites and have not been grown on lifeless media. Biologically not far removed from them is the psittacosis-group, whose members are commonly included in the viruses ; they are the largest of the viruses, up to 300 mμ in diameter, are to some extent antigenically related to each other, seem to have a definite life-cycle within the cytoplasm of animal cells and are, many of them, unlike other viruses, susceptible to the therapeutic action of sulphonamides.

Constitution of Viruses

For a reasoned approach to the chemotherapy of virus diseases a knowledge of the constitution of viruses is essential. There is general agreement on the relative diameters of viruses ; these lie between 10 and 300 mμ. It is also known that the shape of many viruses is very approximately spherical or ellipsoidal. There are great difficulties in obtaining such small particles in a state of purity, with the result that, although their chemical composition approximates to that of cells and bacteria, there is a dearth of exact knowledge on this matter. More is known of the chemical make-up of the viruses of vaccinia (Hoagland, Ward, Smadel & Rivers, 1942), influenza (Taylor, 1944), poliomyelitis (Gard, 1943 ; Loring & Schwerdt, 1942) and equine encephalomyelitis (Taylor, Sharp, Beard & Beard, 1943) than of the others, but each of these animal viruses may be described as a lipoid nucleoprotein complex. On the other hand, according to Janssen (1941) the virus of foot-and-mouth disease is entirely protein. The plant viruses are, so far as is known, exclusively nucleoproteins. Though most of the known animal viruses contain lipoids as integral portions of their structure, that of rabbit papilloma is believed to consist wholly of nucleoprotein (Beard, Bryan & Wyckoff, 1939 ; Taylor, Beard, Sharp & Beard, 1942). The nucleic acids of the plant viruses are of ribonucleic acid type, whereas those of animal viruses are either of ribonucleic acid or of desoxyribonucleic acid type. The presence of nucleic acid implies the presence of bound carbohydrate, but in influenza virus the carbohydrate content is greater than can be accounted for in combination in desoxyribonucleic acid (Taylor, 1944). It has also been found that the lipoids connected with viruses may include fats, phospholipids and cholesterol.

Vaccinia virus has phosphatase, catalase and lipase closely associated with it, but no dehydrogenases (Macfarlane & Salaman, 1938 ; Hoagland *et al.*, 1942), and is itself attacked by papain but not by trypsin, chymotrypsin, ribonuclease or carboxypeptidase (Hoagland, Lavin, Smadel & Rivers, 1940). On the other hand, a staphylococcal phage is attacked by chymotrypsin, but not by trypsin or pepsin (Northrop, 1938, 1939). Vaccinia has also been found to contain copper, and the fluorescence of vaccinia in ultra-violet rays led to the discovery of a flavine-adenine-nucleotide as a component in amount comparable with that in animal cells and some bacteria (Hoagland, Ward, Smadel & Rivers, 1941). As there is no evidence for the growth or metabolism of any virus in artificial media, the function of the constituents of viruses is not capable of direct investigation.

Chemotherapeutic Approach to Virus Diseases

If viruses consist largely of a lipoid-nucleoprotein complex they should be vulnerable to attack. It is known that their activity is destroyed *in vitro* by many bacterial disinfectants and detergents, the latter probably bringing about the disintegration of the liponucleoproteins by setting free the lipoids, nucleic acids and proteins. The protein part is also amenable to attack by denaturants such as urea, guanidine, propylene glycol and potassium salicylate. Such methods of approach to a chemotherapy of virus diseases are however excluded by reason of the intracellular habitat of viruses. The whole difficulty of attacking agents such as viruses and rickettsiae with drugs lies in the facts that their sphere of activity is within the cell or even within the nucleus, and that their metabolism is very closely linked with that of the cell. Similar difficulties probably explain why serotherapy is so ineffective in virus diseases.

We do not know, however, whether a virus may not at times multiply within a limited number of cells, destroy them and, being liberated in the process, go on to invade more cells and destroy them in turn. If this happens, there is at least a possibility of sometimes catching the virus in the open and destroying it with a viricidal drug. It is possibly in some such way that measles antiserum, given during the incubation period of the disease, may suppress or modify its attack. A stimulation of the normal defence-mechanisms of the body against viruses, if it were possible, might be of value at an extracellular stage in the infection. It might also be possible to deny access of the virus to the cell by chemical modification of the cell's surface.

The main problem in the chemotherapy of virus diseases is, however, an attack on the viruses in their cellular environment. This could be a direct attack on the virus itself, or an attack on the sequence of processes involved in its multiplication. It is in the latter aspect that the greatest scope for interference lies. The problem is, however, one of the greatest difficulty, for the virus, having gained an entry into the cell, where dynamic conditions prevail, apparently mobilizes the anabolic processes of the cell for its own repro-

duction, and any selective disruption of this reproductive mechanism without at the same time harming the host-cell presents a formidable task.

What, therefore, are the possibilities of interfering with the reproduction of the virus in its intracellular habitat (Mudd, 1945)? The use of enzymes for bringing about the disintegration of the virus by a lytic action at any link in its structure is excluded in such an environment. Similarly the use of the usual laboratory lytic agents is out of the question. In fact, the only approach seems to be an attack on the "nascent virus". There are possibilities here. On the Woods-Fildes hypothesis it should be possible to inhibit specifically the essential metabolites necessary for reproducing the virus structure by presenting to the cell chemical substances foreign to the cell but bearing a resemblance to the essential metabolites. In this connexion McKinstry & Reading (1944) have examined the effect of a large number of synthetic pyrimidines on the course of experimental poliomyelitis virus in mice, in the hope that compounds of this type might compete with the normal pyrimidine components of the virus for the synthetically-active enzyme systems. No unequivocal success could be recorded. The successful use of derivatives of sulphanilamide for combating infections with some members of the psittacosis group of viruses is, however, an excellent example of this principle of interference. Presumably *p*-aminobenzoic acid is necessary for the reproduction of this group of viruses, and is competitively inhibited by sulphanilamide or its derivatives.

Another possibility of interference is to present to the cell substances foreign to it, in the hope that they may be built up irreversibly into the structure of the virus with the production of something abnormal, in fact, a non-selfreproducing unit. That foreign substances can so be built up is instanced by the fact that wheat and corn grown on seleniferous soils produce proteins containing selenium bound in a form resembling the amino-acid cystine (Painter & Franke, 1935). Andrewes, King & Walker (1946a) have suggested that one interpretation of their finding that *p*-sulphonamidobenzamidine and *p*-sulphonamidobenzamidoxime have a therapeutic effect on an experimental infection of typhus in mice, would be to suppose that these amidines and amidoximes enter the cell and are built up, whilst nucleic acid synthesis is active, into some essential vital structure of the rickettsiae; this might well be through the amidine or amidoxime group, a group whose elements occur once in every pyrimidine and twice in every purine.

Another avenue of approach not covered by the foregoing, would be the discovery of chemical agents which would combine with the substances essential for virus reproduction, or with the enzymes which control the synthetic processes. In the latter connexion, many enzymes concerned in growth contain sulphydryl groups, and it might be possible to block their activity selectively; claims to have achieved this in experimental poliomyelitis in mice with neoarsphenamine and other arsenicals are made by McKinstry & Reading (1945). The recent results of Fitzgerald and his colleagues (Fitzgerald & Babbitt, 1946; Fitzgerald & Lee, 1946) are also instructive. Several acridines, and particularly "phosphine GRN", were capable of inhibiting the reproduction of a coli-bacteriophage at concentrations below the bacteriostatic end-point. The anti-viral action was counteracted by ribonucleic acid, and the suggestion is made that the acridines inhibit the process involved in virus multiplication by competing for some substance related to nucleic acid. The strongly-acidic phosphoric acid groups are salient features of nucleic acids and certain co-enzymes, and it is probable that they are the primary points of attachment of acridines and merit further experimental study *vis-a-vis* other types of bases with substantive character.

Another approach to the whole problem may come through an "interference phenomenon", which is, at any rate superficially, of quite a different nature. Many examples are now known in which infection of a host with one virus may suppress the activity of a second virus added together with, or even before, the "interfering" virus (Andrewes, 1944). Delbrück & Luria (1942) found that a bacterial virus (bacteriophage) inactivated by ultra-violet radiation could thus block the activity of living virus. Similar results were obtained by Henle & Henle (1943, 1944) and Ziegler & Horsfall (1944) for influenza, and by Andrewes & Elford (1946) for ectromelia virus. If the interfering principle, which seems to reside in the killed virus particles, could be isolated, chemically identified and perhaps imitated, new chemotherapeutic possibilities would be opened up. It might, however, be that an interfering agent was thus obtained, not so very different in its actions from those visualized in an earlier paragraph.

Results of Chemotherapeutic Trials

a. Rickettsiae. Though passing mention has been made of several of them, more detailed attention must now be given to such few successes as chemotherapy has already had in the field of rickettsiae and viruses. Activity against experimental typhus in mice has been found in the last few years in compounds of several unrelated groups of chemical substances. While the sulphonamides in the usual clinical sense seemed to do more harm than good, *p*-sulphonamidobenzamidine and the corresponding amidoxime were found to be active (Andrewes, King, van den Ende & Walker, 1944); closely-related compounds had lesser degrees of activity, but very little modification of the molecule was needed to abolish activity altogether.

The drugs had no *in vitro* action on the rickettsiae; possible mechanisms for their *in vivo* action are discussed by Andrewes, King & Walker (1946a). Their action was not inhibited by *p*-aminobenzoic acid. This latter substance has itself been claimed to have chemotherapeutic value against typhus both in man and in mice (Yeomans, Snyder, Murray, Zarafonetis & Ecke, 1944). Activity against infection in mice has also been found in methylene-blue (Kikuth & Schilling, 1944), toluidine-blue and "forbisen" (Peterson, 1944), several dyes related to methylene-blue (Andrewes, King & Walker, 1946b) and penicillin (Moragues, Pinkerton & Greiff, 1944).

The dyes, unlike the compounds mentioned earlier, had a direct inactivating action on the rickettsiae, demonstrable *in vitro*. Activity of toluidine- and methylene-blue and of *p*-aminobenzoic acid has been shown against other rickettsial infections, those of rocky mountain spotted fever, and of scrub typhus (Hamilton, 1945; Murray, Zarafonetis & Snyder, 1945; Anigstein & Bader, 1945). All these findings are at present of interest rather to the student of the theoretical aspects of the subject than to the practising clinician.

b. Psittacosis group. There is general agreement that a number of viruses in this group are susceptible to chemotherapeutic attack with sulphonamides or penicillin. Those of lymphogranuloma venereum and mouse-pneumonitis are readily attacked by a number of drugs in the sulphonamide group, though different authors place the various drugs in a

different order of effectiveness (Findlay, 1940b ; Jones, Rake & McKee, 1941 ; van den Ende & Lush, 1943 ; Felton, Hebb & Oliphant, 1943). The substances prevent death from lymphogranuloma in mice, but rarely cause complete sterilization of the infection. Most workers agree that they have no *in vitro* killing action and are inhibited by *p*-aminobenzoic acid (MacCallum & Findlay, 1938 ; Levaditi & Perault, 1942 ; Rodaniche, 1943 ; Findlay, 1940a ; Mudrow & Bock, 1943), but a few workers have put forward contrary views on these two points (Seeler, Graessle & Dusenberg, 1943 ; Holder, Levine & Bullowa, 1942). Eaton & Hanford (1945) report very different activity on the part of sulphamerazine according to the host (mouse, hamster or chick embryo) in which the drug was tested. Some viruses of the group, when given intravenously in high concentration, kill within 36 hours by a so-called " toxic " effect. Such toxic deaths were not prevented by sulphonamides (Rake & Hamre, 1944).

Other viruses of this group, those of psittacosis, meningopneumonitis, and feline pneumonitis, seem to be unaffected by sulphonamides, but it is uncertain whether this will prove to apply to all strains, or whether there are strain differences in sensitivity to these drugs such as exist, for example, among streptococci. Trachoma and inclusion-conjunctivitis in man are held to be benefited by sulphonamides ; here some part may be played by the action of the drugs on associated bacteria (Loe, 1938 ; Barrat, 1941). Heart-water of cattle responds to a sulphonamide, uleron (Neitz, 1940) ; though usually classed as a rickettsia (*R. ruminantium*), the agent of this disease is probably better placed with the group now under discussion. While not, or but rarely (cf. Bedson, 1943), affected by sulphonamides, the psittacosis virus can be attacked successfully with penicillin, at least in experimental infections (Heilman & Herrell, 1944 ; Parker & Diefendorf, 1944 ; Bedson & May, 1945), though it is likely that the dosage required is outside the limits practicable for man. Finally, Mauer (1938) has reported activity on the part of trypaflavin against psittacosis in mice.

c. Action of sulphonamides on other viruses. Claims have been made for activity on the part of various sulphonamides against influenza, lymphocytic choriomeningitis, dog distemper, measles and smallpox. These claims have failed of confirmation in the case of influenza virus and choriomeningitis infections. Activity in dog distemper (Marcus & Necheles, 1938 ; Bryan, 1941), measles (Hogarth, 1939 ; Anderson, 1939) and smallpox (Patel & Naidu, 1940 ; Cottrell & Knight, 1943 ; Leishman, 1944) is almost certainly due to an effect on the secondarily-invading organisms which are responsible for most of the complications and mortality. Evidence of action against the viruses themselves is lacking. Remarkable potency of sulphonamides against a virus associated with fowl-paralysis has been reported by Asplin (1944), but no confirmation by other workers has appeared.

d. Other chemotherapeutic studies in virus infections. Negative results of trials have been reported by numerous workers. Reference need be made only to those papers which report tests of considerable series of compounds : on influenza (Coggeshall & Maier, 1942 ; Krueger *et al.*, 1943 ; Andrewes, King & van den Ende, 1943), on poliomyelitis (Coggeshall & Maier, 1942 ; Kramer, Geer & Szobel, 1944 ; McKinstry & Reading, 1944, 1945), on lymphogranuloma venereum (Andrewes, King & van den Ende, 1943), on St. Louis encephalitis (Kramer, Geer & Szobel, 1944), and on typhus (Andrewes, King, van den Ende & Walker, 1944) ; (see also van den Ende, Stuart-Harris, Fulton & Niven, 1946).

Of claims to have achieved success, few are based on strong evidence. Mention may be made, however, of reported activity of mercurochrome against canary-pox (Manwell & Goldstein, 1939 ; Coulston & Manwell, 1941) and of quinine against fowl-pox (Robbins, 1942). No attempt will be made here to review the numerous studies on *in vitro* action of many compounds on viruses, for it is questionable how far the results obtained are likely to find application in *in vivo* trials.

e. " Chemotherapy " of bacteriophage. A number of substances have been shown to be capable of suppressing the activity of a bacteriophage without greatly affecting the growth of the bacteria it attacks. While there is dispute as to how far analogies to infections with animal viruses may usefully be drawn from such studies, some of the results are of much interest and may possibly furnish useful clues. Stassano & de Beaufort (1925) and Bordet & Renaux (1928) found that some phages could be inhibited by dilutions of citrate and oxalate which did not affect the associated bacteria ; the phage apparently needed more calcium ions for its growth than did the bacteria. According to Wahl (1946), certain phages require an optimum concentration of calcium for lysis, but lysis can be produced with less than the optimum amount of calcium ions if aneurin is added. Spizizen (1943) found that arsenite acted as a specific inhibitor of a coli-phage (γ). Reference has already been made to the work of Fitzgerald *et al.* (1946) on similar activity on the part of " phosphine GRN ".

* * *

Though the whole problem is a difficult one, there exist leads which are not without promise ; of greatest encouragement is the fact that the rickettsiae and some of the larger viruses are susceptible to chemotherapeutic attack although they are, like the smaller viruses, strict parasites, closely dependent on the conditions existing within the cell for their life and growth.

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PRINCIPLES AND PRACTICE OF LOCAL CHEMOTHERAPY

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By some the word " chemotherapy " is confined to the use of substances having a systemic action, but by its literal meaning it should include the effective use of these and other substances by local application. Although the most notable advances of the past decade have been in the field of systemic treatment, there has also been a remarkable improvement in the results obtained by applying substances with an antibacterial action to the site of infection itself. Indeed, it is only within the last six years—a period almost coinciding, not without reason, with the World War—that this kind of treatment has been developed on rational lines. This change has been due in part to the introduction of new and more powerful antibacterial agents, but also to the employment both of these and of older remedies in a more intelligent way. These advances have been connected mainly with the treatment of various kinds of wound, but infections in other parts of the body, such as the skin and various mucous membranes, have not been neglected.

PRINCIPLES

If local chemotherapy is to succeed, certain conditions must be fulfilled, and it is easy to illustrate failure due to lack of their observance by examples from the past. The chief of these conditions are the following:

Adequate Antibacterial Action

It goes without saying that the substance employed should be capable, at least in favourable circumstances, of killing or at least of preventing the growth of the micro-organisms concerned. An example of a frequently-used wound antiseptic almost devoid of this power is iodoform. It is particularly interesting that this was the only wound antiseptic applied in the past in the form of a powder. This method has recently been recognized as having great advantages—provided, of course, that the substance so used has the necessary action—because it ensures persistence of effect.

It is often necessary to know the precise nature of an infection before deciding how to attack it, because the susceptibility of different micro-organisms to the same agent

may vary greatly. Generally speaking, the better the agent the more selective is its effect. Phenol is a weak germicide, but being simply a general protoplasmic poison, acts more or less equally on all bacteria. Acridines and dyes in general have a highly selective action, exerted mainly against gram-positive bacteria. The acme of selectivity is reached in penicillin, with an unexampled action on some bacterial species and less than none on others. I say less than none for two reasons : some bacteria are not only unaffected by penicillin but produce an enzyme which destroys it ; secondly, there is some evidence that certain concentrations of penicillin actually stimulate the growth of bacteria resistant to it.

Efficacy in Body Fluids and Tissues

It is not enough that the agent used is lethal to bacteria in the test-tube ; it must retain this power in the environment in which it is required to act in the body. This may be mucous secretion, faeces, blood, a serous effusion or pus. It is well recognized that many antiseptics fail on this account ; magnificent performers in water, they lose all activity in a wound. This is due to chemical combination with some constituent of the medium, and is best illustrated by oxidizing agents such as hydrogen peroxide and potassium permanganate, and the halogens. Iodine and chlorine are both rapidly inactivated by body fluids ; iodine, although an excellent skin disinfectant, is therefore not an efficient wound antiseptic.

The more modern local chemotherapeutic agents owe their success largely to the fact that such obstacles do not deter them. In the first full description of the properties of penicillin (Abraham, Gardner, Chain, Heatley, Fletcher, Jennings & Florey, 1941), experiments were described showing that its action on bacteria was exerted equally in broth, serum, blood and even pus. It seems likely that streptomycin will not prove equally indifferent to its environment : Abraham & Duthie (1946) have shown that its activity is much diminished in acid media. Since an acid reaction is often developed in foci of infection, the action of streptomycin may be hindered where it is most needed.

The Use of a Suitable Vehicle

This condition must be inserted because preparations have been used from which the active substance is not properly liberated. An extreme example of this is the old practice of dissolving phenol in olive oil ; the resulting product was popular because it had none of the caustic action of a watery solution of the same strength. The explanation was simple : there was no caustic action because no phenol escaped from the oil, and for the same reason no effect on bacteria can have been exerted. The same objection applies, although not completely, to suspensions in liquid paraffin and other mineral-oil bases ; these are generally less effective than watery solutions, although for some purposes they have other advantages.

Penicillin is frequently used in the form of an ointment or cream. It is important to know that the base is suitable. It must neither destroy the penicillin, nor retain it when the preparation is in contact with the tissues. Selbie, Simon & McIntosh (1945) have condemned a form of penicillin cream commonly used in Great Britain for failure to liberate penicillin freely ; others have found this preparation more satisfactory.

Adequate Distribution

The agent used must be brought into contact with the whole of the infected area. In the treatment of superficial infections this presents no difficulty ; in treating deep wounds or long sinuses, the difficulty may be insuperable. It is impossible, at least by ordinary methods, to disinfect a deep penetrating wound which has traversed muscle and fascia. Many wounds due to bullets and shell-fragments are of this nature, and it is therefore not surprising that applications to such wounds have often failed to prevent sepsis.

Adequate Persistence

All the most effective agents for local chemotherapy act slowly on bacteria. It is therefore necessary that they should be enabled to act for at least several hours and perhaps longer. This cannot often be achieved by the use of a solution, which was almost the only method used in the past. Antiseptics have often been used in so perfunctory a manner that only magical powers could have enabled them to have any effect. There has been a certain ritual which surgeons have been accustomed to perform, not unlike the rite of baptism, since the effect, if any, can only have been supernatural.

Two methods have largely superseded the use of solutions. One is the use of semi-solid preparations, with which a wound or other cavity is filled, after which it is covered with an occlusive dressing. Propamidine has been used in this way in the form of a jelly (Thrower & Valentine, 1943). Meleney's (1939) zinc peroxide paste is similarly applied, and a semi-solid cream of penicillin was successfully used by Florey & Williams (1944) for packing septic wounds of the fingers and hand. Perhaps the more important innovation is powder treatment. Here the agent is applied in solid form, and dissolves slowly in the exudate, thus maintaining an adequate concentration for some length of time. The first substance to be used in this way was iodoform, for the simple reason that it is almost completely insoluble in water, and hence could be used in no other way. Modern and effective powder treatment dates from the introduction of sulphonamides as local applications, which followed several years after their first use as systemic chemotherapeutic agents. Penicillin and acridine compounds (usually proflavine) have also been used in powder form, sometimes undiluted, but more often diluted with sulphanilamide or sulphathiazole.

The rate of solution of an agent used in this way is important. As Hawking (1941) showed experimentally, sulphanilamide is too soluble and sulphapyridine too insoluble for this purpose ; one dissolves and disappears in too short a time, and much of the other may still be undissolved after several days. Sulphathiazole has an intermediate solubility and is thus more suitable, either alone or in combination with sulphanilamide, the latter giving a useful high initial concentration. It would be useful for this purpose if penicillin were a less soluble substance ; proflavine (solubility 1 in 300) is ideal, persisting in adequate concentration for a long period.

Accessibility of the Infection

Local chemotherapy can be expected to succeed only in superficial infections, or in infection of cavities. If deep penetration of tissue is required, none but the systemic route is of any avail. It seems scarcely necessary to point this out, but gas gangrene was frequently treated in the past by the local application of hydrogen peroxide, than which

nothing could have been more futile. In this disease, the infection has spread into muscle many centimetres from the wound-cavity before the diagnosis is made. Inaccessibility may also result from loculation in cavities, and from the presence of sloughs, necrosed bone or other non-viable tissue.

Effect on Tissues

It is almost axiomatic that most ordinary antiseptics do as much damage to tissues as to bacteria. The ideal local chemotherapeutic agent should, as far as possible, be free from this disadvantage. The least toxic of all is penicillin, which has practically no ill effect on the cells of any tissue even in concentrations much higher than those which it is necessary to use. The sulphonamides are also comparatively harmless, but in concentrated solution they diminish cellular activity, and nervous tissue is particularly susceptible to damage by them. The least toxic of the older antiseptics are the acridine compounds, and although in concentrated form they cause actual necrosis of certain tissues, they can be so used as to obviate this effect. These are the three agents which have been chiefly used in the recent treatment of wounds.

PRACTICAL APPLICATION

1. Wound Prophylaxis

The prevention of wound sepsis is perhaps the most important of all forms of local chemotherapy. A clear distinction must be made between the prevention of wound infection and its treatment. For about two hours after the infliction of the wound, contaminating bacteria are still in the cavity of the wound, and have not begun to multiply and invade surrounding tissues. They are thus still relatively accessible, although those enmeshed in blood-clot, or situated in crevices between planes of tissue, are not likely to be killed by any application which has only a transient effect. No such application can take the place of necessary surgical treatment ; foreign bodies and devitalized or grossly contaminated tissue must be removed.

The ineffectual attempts to prevent wound sepsis in the war of 1914-18 are now of only historical interest ; their failure was due to delay in treatment, inadequate surgery, and the use of unsuitable agents, chiefly the older antiseptics with a high tissue-toxicity. The intelligent use of more effective agents dates from 1939.

Sulphonamides. The powder treatment of wounds with sulphonamides was introduced by Jensen, Johnsrud & Nelson (1939) for the treatment of compound fractures in civilian practice. The method was widely employed for the initial treatment of battle-wounds in the war which began in that year. We have very little statistical information about its effect, although there is experimental evidence in plenty (Stephenson & Ross, 1940 ; Henderson & Gorer, 1940 ; Hawking, 1941 ; McIntosh & Selbie, 1942) of its efficacy under certain conditions in the prevention of gas gangrene.

Of its effect in preventing sepsis generally the clearest evidence is that collected by Bentley & Thomson (1945) among casualties during the battle for the Gothic Line in Italy. Their best results were obtained with penicillin, but in two other series of wounds, one treated with sulphanilamide, and one untreated by any form of local chemotherapy, the percentages becoming septic were 11 and 23 respectively. The percentages " infected " in the sense of containing pyogenic cocci without exhibiting signs of gross sepsis were 43 and 49 ; the authors conclude that sulphanilamide often restrains bacterial activity sufficiently to prevent sepsis, but has little power of eradicating infection altogether. In the later stages of the war penicillin was used so extensively that further information about the action of sulphonamides alone has been scanty.

In blood or serous exudate the sulphonamides have a bacteriostatic action on pyogenic cocci and on some of the *Clostridia* and, provided that the powder is adequately distributed, and that solution and absorption are neither too rapid nor too slow, the multiplication of such bacteria must be restrained. If the method has been abandoned for some purposes, it is because even more effective methods are now available. The only serious evidence against its efficacy is the analysis of results in 2,191 civilian cases by Meleney (1945) according to which sulphonamide treatment, either systemic or local, did not reduce the frequency of infection at all. The discrepancy between these results and most others has yet to be explained.

Acridine compounds. The subject of acute controversy since 1917, when they were first introduced, the acridine compounds have now found their proper place in wound treatment. Without reviewing their full history, it may be said that they owe their present recognition largely to the work of Albert and his colleagues, who have been indefatigable in synthesizing and studying the properties of new acridines, and in defining the conditions of their employment. It is due to their influence that acriflavine, which was largely used in earlier days, has been discarded ; this substance is inconstant in composition, too toxic and too soluble. Proflavine, on the other hand, is a pure substance, of the ideal degree of solubility for persistent action when used in powder form, and less toxic. Perhaps the greatest influence of any single paper on this subject has been exerted by Russell & Falconer's (1941) observation that a buffered isotonic solution of proflavine is almost without toxic effect on an exceedingly vulnerable tissue, the exposed surface of the brain.

No one questions the antibacterial activity of the acridine compounds ; their *in vitro* activity is very great and their capacity for preventing infection has been demonstrated repeatedly. They were found more effective than sulphonamides in two series of observations already quoted, those of Hawking (1941) and of McIntosh & Selbie (1942) on the prevention of gas gangrene. Recent controversy has centred chiefly on their toxicity when used in solid form. There is no doubt that proflavine powder in any considerable quantity will cause necrosis in normal tissues ; Hawking (1943), Russell & Falconer (1943) and Russell & Beck (1944) have demonstrated this in various ways, using the powder either pure or diluted. Selbie & McIntosh (1943), on the other hand, consider that the changes produced by a powder containing 1 part of proflavine to 99 of sulphathiazole are not such as to contraindicate its clinical use. This mixture has been extensively used for the prevention of sepsis, and favourably reported on by McIntosh & Selbie (1944) and Ascroft (1944).

It was suggested by the writer (Garrod, 1940) that punctured wounds into which an antiseptic cannot easily be introduced might be treated by infiltrating the surrounding tissues with a solution of an acridine compound. This technique has been employed clinically by Arden (1945), using a 0·1% solution of 5-aminoacridine hydrochloride, without evidence of any undue toxic effect ; the results in preventing sepsis were considered good.

Penicillin. Against certain bacteria, penicillin is by far the

most powerful antiseptic known, and its absence of toxicity is another unique property. On the other hand, its free solubility is a drawback, since persistence following powder application is likely to be short. Penicillin became available in such vast quantities in the later stages of the war that nearly all our information about its power of preventing sepsis in wounds refers to parenteral treatment. It is nevertheless evident from the original studies of Florey & Cairns (1943) and from those of Bentley & Thomson (1945) that calcium penicillin, diluted with sulphanilamide or sulphathiazole, and used by insufflation, is a valuable prophylactic.

It is very desirable that further studies of the usefulness of penicillin-sulphonamide powders as wound prophylactics should be made. It has yet to be decided what is the best method of dealing with the various types of wound encountered in civilian practice, and present evidence suggests that such a powder is likely to be as effective as any other single application in preventing the development of infection.

2. The Treatment of Wound Sepsis

The treatment of a wound which is already suppurating is an entirely different problem from the foregoing. Large numbers of bacteria are present, in a different medium, and some are in the tissues bordering the wound. If the surrounding tissues are being invaded deeply, as in a spreading cellulitis or gas gangrene, local chemotherapy is of little value. The infection must be localized to the wound and, since it is usually suppurative, the chosen agent must be active in the presence of pus. That they are inactivated by tissue-breakdown products in pus is the reason for the relative uselessness of the sulphonamides for this purpose.

The acridines have no such disadvantage, and their successful modern use in septic wounds dates from the introduction of proflavine powder treatment by Mitchell & Buttle (1942). Granulation tissue is far more resistant than the normal tissues bordering a fresh wound to the toxic action of proflavine, and these authors introduced quantities up to 2 g. of the undiluted powder without ill effect, although amounts less than this were usually adequate. Wounds so treated dried up quickly and began to heal, even though suppuration had previously continued for months. This treatment has also been used successfully by Raven (1944) and by Heggie, Warnock & Nevin (1945). Mitchell & Buttle (1943) subsequently used diflavine (2 : 7-diaminoacridine monohydrochloride) in the same way with good results, and continuous irrigation with a solution of this compound or of 5-aminoacridine is recommended by Poate (1944). McIntosh, Robinson & Selbie (1945) report favourably on flavazole, a chemical compound of proflavine and sulphathiazole.

It is impossible here to review the many uses of penicillin in treating sepsis in various types of wound. Both powder insufflation and the instillation of a solution after suture were found by Florey & Cairns (1943) to control all gram-positive infection remarkably, at least in wounds not more than ten days old. Parenteral treatment has since very largely displaced these methods. How to treat septic conditions of the hand by locally-applied penicillin in the form of powder or paste is admirably described by Florey & Williams (1944). Provided that the infection is caused by penicillin-sensitive bacteria, that the whole area is accessible, and that persistence is achieved, this treatment is more successful than any other.

It is impossible to exaggerate the importance of technique in local penicillin therapy; progress has indeed consisted entirely of devising new methods of application for particular conditions. The chief aim of these has been to secure persistence of effect without repeated application. The latest example of these ingenious methods is that devised by Reading (1946) for mastoidectomy wounds. Florey & Florey (1943) originally suggested, for this purpose, suture of the wound and repeated instillation of penicillin solution through a tube. Reading fills the cavity with plasma in which penicillin has been dissolved, coagulates this by adding thrombin, closes the wound, and leaves it undisturbed until the sutures are removed. Primary healing resulted in 46 out of 50 cases. The plasma clot acts as a depot from which penicillin diffuses uniformly, presumably for as long as it is needed.

Penicillin is useless for "gram-negative" infection, due to such bacteria as *Proteus* and *Ps. pyocyanea*. These bacteria are also very resistant, although not completely so, to the acridines. Good effects on these infections have been claimed for ethylene glycol monophenyl ether (phenoxetol) (Berry, 1944; Gough, Berry & Still, 1944).

The place of cationic detergents in the treatment of wounds is still uncertain. They have a marked cleansing action, and probably have considerable prophylactic, although not much therapeutic, value. Examples are zephyran (Schumacker & Bethea, 1943), cetavlon (Barnes, 1942; Williams, Clayton-Cooper, Faulkner & Thomas, 1944) and phemeride (Hand, 1944).

3. Infections of Closed Cavities

The treatment of meningitis, empyema and suppurative arthritis has been revolutionized by the advent of penicillin. Provided that the infection is due to a penicillin-sensitive organism, the injection of penicillin solution into the infected cavity is the essential feature of the treatment. This is both far more effective and more economical than parenteral injection. The anatomy of the lesion fulfils one of the main conditions for success in local chemotherapy—that action shall be persistent. A single injection into such a cavity, where the solution is wholly retained and loss can result only from absorption, maintains its effect for many hours and, in the infected pleura, even for several days.

This form of treatment is applicable to meningitis, empyema, pericarditis, arthritis, and to abscesses in other situations such as the breast or connective tissue generally. It does not necessarily render ultimate evacuation of an empyema, or of the contents of an abscess elsewhere, unnecessary, even though the contents may be sterilized.

4. Skin Disease

Superficial bacterial infections of the skin offer a promising field for local chemotherapy, and a variety of applications used in the past probably had some such effect. They have now been largely replaced either by sulphonamides, which are undoubtedly effective, at least in impetigo, or by penicillin. There is now a considerable literature on the treatment of skin diseases with applications of penicillin in the form of a spray or cream, which it is not proposed to review here. The results can be summed up very briefly: When the primary cause of the disease is infection by staphylococci or streptococci, the treatment succeeds; when secondary infection by such organisms is present, there is improvement; when such infection plays no part in the disease, there is no

effect. The local treatment of blepharitis and conjunctivitis has also been highly successful when a penicillin-sensitive infection is the cause.

It is interesting that the conjunctival sac has been the site of almost the only example of chemoprophylaxis on which reliance has been placed throughout years of scepticism about antiseptics. The instillation of silver nitrate solution into the eyes of new-born infants has presumably prevented many cases of ophthalmia neonatorum. The time has come for this solution to be replaced by something less irritating and perhaps more effective. The application of penicillin cream to the eyelids would fulfil these requirements.

5. The Respiratory Tract

The gargles, sprays, inhalations and lozenges of the past can have had extremely little effect. Not only were most of the antiseptics contained in them ineffective in themselves, but the method of application was much too transient. The factor of persistence is as important here as anywhere else, and none of these measures secured it. Effective local chemotherapy in the upper air passages began with the introduction by Delafield, Straker & Topley (1941) of sulphathiazole snuff for the treatment of nasal carrier-states. The slow solution of this powder in the nasal secretion was calculated to produce an effect lasting several hours. This treatment has been successfully used in treating carriers of *Staphylococcus aureus* and *C. diphtheriae*.

The more difficult problem of maintaining chemotherapeutic action in the mouth and tonsillar area was first solved by McGregor & Long (1944), who employed gelatin pastilles containing penicillin. These dissolved slowly, and patients were instructed to introduce another as soon as the previous one had disappeared; the effect could thus be maintained throughout the day. This treatment had a remarkable effect on Vincent's gingivitis; it was also valuable in preventing sepsis after dental extraction, compound fractures of the mandible, and tonsillectomy. It afforded some relief in acute streptococcal tonsillitis, and shortened the duration of the carrier-state thereafter. Recently Meadley & Barnard (1946) have advocated the treatment of acute streptococcal and Vincent's infection of the throat by the use of penicillin-sulphathiazole snuff. The medicament is carried through the naso-pharynx to the throat, which it reaches within a few minutes; this was proved both by tests with dyes and by estimating the penicillin content of the oro-pharyngeal secretion, which usually remained therapeutically adequate for 3 hours after taking the snuff.

Finally, penicillin administered by inhalation has been used for the treatment of infections of the bronchi and lungs. This treatment is still in the experimental stage, and exact indications for it have not yet been defined. Marked, although temporary, benefit has been obtained even in bronchiectasis. The treatment of nasal sinusitis has been less successful, perhaps because adequate distribution and persistence cannot be achieved in the complex area involved.

COMMENT

This brief summary has dealt only with local chemotherapy by direct application. Urinary and intestinal antiseptics are in a somewhat different category, although they are in a sense examples of local chemotherapy, and have been the subject of great advances during the past few years. No reference has been made to antibiotics other than penicillin, although it is likely that in the future some of these will be found to have special indications. The most firmly established of these at present is tyrothricin. Suitable, owing to its systemic toxicity, only for local application, this substance has a variety of uses in the treatment of superficial infections, including wounds, ulcers, and infections of the eye and nose. Strange as it may seem, an antibiotic derived from a mould may yet prove to be a useful remedy for superficial mycoses; the studies of Sanders (1946) offer promise in this direction.

Advances in systemic chemotherapy have been the main feature of medical progress in the past ten years. One of their effects has been to revive interest in local chemotherapy. Surgeons and others who would not previously admit that antiseptics had any real value, faced with the spectacle of drugs which actually killed bacteria in the tissues and even in the blood-stream, were compelled to admit that a direct attack upon bacteria in some local and accessible site might also have some effect. The success of such proceedings has been due largely to the advent of new and better chemotherapeutic agents, notably penicillin, but it has depended also on observing principles which it has been the endeavour of this article to define.

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PRINCIPLES OF ADMINISTRATION IN CHEMOTHERAPY

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The successful use of drugs depends on maintaining an adequate concentration at the site of action for an adequate time. The achievement of this aim depends to a very considerable extent on the method of administration, the frequency of administration, and the use of a preparation dispensed in an appropriate form. In the past these matters have been left largely to chance. The therapeutist has decided what he considers is likely to be the best way of giving the drug, and if his results have been good his decision has been justified, but if his results have not been good, it has been difficult to know the reasons for his failure.

In recent years much more attention has been devoted to these matters. Different methods of administration have been controlled by estimates of the concentration of the active substances in the body and this has led to rapid advances. The new drugs which have been introduced recently would have been much less successful if their practical application had depended solely on the old empirical method of trial and error.

The problems involved in the study of the effects of different methods of administration are general pharmacological problems, and various different types of drug have been used in their study, but the present article is concerned primarily with chemotherapy and examples will be chosen, as far as possible, from experiments on drugs used against infections of various kinds. A fuller discussion has been given elsewhere (Gaddum, 1944a).

Methods of administration may be either systemic or local. When a systemic method is used the drug is intended to act after it has been absorbed into the blood-stream and distributed about the body. Such methods are used when the infection is widespread or when the site of infection is inaccessible. One advantage of local administration is that it is more economical, since smaller quantities of drug are generally used. It is also sometimes less dangerous, since high concentrations can be maintained locally which would have toxic effects in the body as a whole. It is also sometimes possible by local administration to reach parts of the body which are inaccessible to drugs given systemically, either because the circulation is poor or for other reasons. When the circulation is good where the drug is expected to act, local administration is seldom effective because the drug is rapidly absorbed into the blood and carried away. In such cases the drug may act both systemically and locally and this may be an advantage, but the therapeutist should know what is happening, and for this reason it is sometimes desirable to estimate the drug in the blood even when it is applied locally near its site of action.

Excretion and Fate in the Body

The optimal frequency of dosing, or rate of administration, depends on the rate of disappearance of the drug from the site of action. This can seldom be measured directly. It depends partly on the rate at which the drug is destroyed locally and partly on the rate at which it is taken up by the blood or lymph or washed away in exudations or secretions. With systemic administration, satisfactory cures are usually obtained if an adequate concentration is maintained in the blood. The disappearance of the drug from the blood depends on the destruction of the drug, on its uptake by various tissues, and on its excretion in the urine, faeces, etc. The relative importance of these factors varies widely. Penicillin is mostly excreted in the urine, mepacrine is taken up by the tissues and slowly destroyed; only a small percentage appears in the urine. Ether is taken up by the tissues and then excreted unchanged. Acetylcholine is rapidly destroyed both in the tissues and in the blood itself.

The fate of a drug in the body can sometimes be followed by measuring the amounts in various organs and excreta, but it is seldom possible to account for the whole dose in this way. The simplest way of studying the relative importance of destruction and excretion is to eliminate complications due to the transference of the drug from one organ to another by making an extract of the whole animal. This type of experiment is most easily done with small animals such as mice or rats. Alexander (1943) carried out such experiments with sulphanilamide. Experiments on rabbits showed that this drug was first evenly distributed in the body-water and then concentrated in the liver, kidneys and urine, but a certain proportion of the dose could not be accounted for either in its original form or as the acetyl derivative. The suspicion that some of the drug had actually been destroyed was confirmed by estimations made on extracts of whole mice, which showed that 30% of the drug was destroyed and the rest was mostly excreted in the urine.

Frequency of Dosage: "Halving Time"

The curve showing the disappearance of drugs is often approximately exponential; the concentration falls rapidly at first and then gradually more and more slowly. The rate is conveniently measured by the half-disappearance time, or halving time, which is the time taken for the concentration to fall to half its initial value. When penicillin, diodone or *p*-aminohippuric acid is given intravenously, the halving time is measured in minutes. The halving time for sulphonamides or salicylates is measured in hours; for mepacrine or digitalis in days; and for suramin or mercury in weeks.

The frequency of dosing should generally not be less than the halving time if the concentration is to be kept fairly constant. The administration of larger doses at longer intervals may keep the concentration above the effective level, but is a comparatively wasteful way of using the drug, and is generally more liable to cause toxic effects. The more frequent the dosing, the more constant the concentration, but the greater the labour. The optimal frequency depends on a balance of these two factors. Continuous administration is ideal both for economy of drug and constancy of concentration. It can be achieved by an apparatus which produces a steady flow of solution or by the more or less steady liberation of the drug from a more or less insoluble depot. Examples of these techniques, both for systemic and local administration, will be given later.

Devices which slow the disappearance of a drug have two advantages. They decrease the amount needed and make it easier to attain a steady concentration. The appropriate method depends on the mode of disappearance. Acetylcholine is destroyed by cholinesterase, and its disappearance can be slowed by giving eserine, which inhibits this enzyme. When a drug is excreted in the urine, its disappearance can sometimes be delayed by slowing the excretion. The great speed at which penicillin appears in the urine proves that it is actively excreted by the tubules. Its disappearance can be slowed by giving other drugs which are excreted in the same way, and so overload the excretory mechanism. Both diodone (Rammelkamp & Bradley, 1943) and *p*-aminohippuric acid (Beyer, Woodward, Peters, Verwey & Mattis, 1944) have this effect.

When a drug is given continuously, or in frequent small doses, it gradually accumulates in the body, and the half-accumulation time is equal to the half-disappearance time (Gaddum, 1944b). When the having time is short, as with penicillin, the concentration rapidly rises to the desired level, but when the halving time is longer the concentration may rise too slowly, and it is necessary to give large doses at first and then reduce the rate of administration when the effective concentration is attained. This technique is used with digitalis, salicylates, mepacrine, sulphonamides and many other drugs which are given systemically. It is not commonly used for local applications, because in this case the halving time is generally short.

The dose necessary to produce a given initial concentration in the blood depends on the volume of distribution. The dye T1824, or Evans blue, is used to measure the plasma-volume, which is equal to its volume of distribution. With chlorides, bromides and thiocyanates the volume of distribution is roughly equal to that of the extra-cellular fluid. Many substances, such as alcohol, urea or sulphanilamide, are evenly distributed in all the body-water. Some drugs, such as mepacrine, are rapidly concentrated in certain tissues, and the apparent volume of distribution is much larger than the total volume of the body. The importance of these facts is mainly theoretical, and the appropriate initial dose is determined empirically.

Oral Administration

The simplest way of taking drugs is to swallow them, and this is the method of choice when it is effective, but many drugs are poorly absorbed or destroyed before absorption. Absorption through the oral mucous membrane is slow and, although some drugs have systemic effects when given in lozenges or pastilles to be placed under the tongue, this technique is mainly used for its local effect in the mouth. For example, penicillin pastilles have given encouraging results in the treatment of Vincent's angina (MacGregor & Long, 1944). Few drugs, except alcohol, are appreciably absorbed in the stomach, and absorption from the rectum is also slow and erratic. The main site of absorption is the small intestine, and various devices have been used to get unstable drugs into the duodenum before they are inactivated in the stomach. The devices that have been tried include duodenal tubes, which are effective but laborious, the administration of alkalis to neutralize the gastric juice, and the use of insoluble preparations or of tablets coated with insoluble materials such as salol, stearic acid, keratin, or gelatin hardened with formalin. Cellulose acetate phthalate has recently been recommended for this purpose. It is soluble in alkalis but not in acids, and experiments with *x* rays and capsules containing barium sulphate showed that disintegration occurred in the small intestine (Hodge, Forsyth & Ramsey, 1943). Such preparations are best given on an empty stomach with a drink of water, so that they pass quickly through the pylorus.

Absorption from the intestine is seldom a very rapid process and drugs given in this way have a more prolonged action than when they are injected. Attempts to prolong their action by the use of insoluble preparations are limited by the fact that drugs which are not absorbed fairly rapidly are apt to be lost through the anus. Various sulphonamide derivatives are comparatively slowly absorbed and have been used to disinfect the lumen of the intestine. They are extremely effective for this purpose and liable to cause vitamin deficiencies in the patient by inhibiting organisms which form vitamins in his gut. The reasons for their slow absorption are uncertain ; they are not all of them particularly insoluble. It is possible that the slowness of absorption of these drugs is due to the fact that they are not ionized (Krebs & Speakman, 1946). On the other hand, it is possible that light will be thrown on this problem by experiments of the type carried out by Bose, Ghosh & Rakshit (1946), who injected various sulphonamides and then estimated their concentrations in the contents of the alimentary canal. Those concentrations were sometimes much higher than the concentrations in the blood. The best drug for disinfecting the gut may be the one which is most actively excreted into the gut. On the other hand, Hawking (1942) obtained evidence that very little sulphaguanidine is excreted into the gut compared with the amounts in the urine. Further evidence is desirable on this point.

Intravenous Injection

Intravenous injections are used to get a quick action, or when the solution is irritant. Rapid dilution by the blood diminishes the irritant effects, though very irritant solutions may cause local phlebitis. If the solution is injected very rapidly the drug may all reach the heart and lungs almost undiluted and cause immediate toxic effects upon them. If a minute or two is taken over the injection, this particular danger is generally avoided, but even then the concentration in the blood as a whole may rise to dangerous levels before the drug passes to the tissues. The dangers of " speed shock " were emphasized by Hirschfeld, Hyman & Wanger (1931). This led to the administration of neoarsphenamine, and later mapharside, by slow intravenous infusion (Hyman, 1940).

The rate of disappearance is usually high when drugs are given intravenously, so that slow, rather than quick, injection is advisable, not only because it is less likely to cause toxic effects, but also because it is more likely to maintain an effective concentration in the blood for an adequate time. Such infusions may have to be maintained for a number of days. Special needles have been devised which can be kept in position on a patient's arm and which give him some freedom of movement (Herrell, 1945), but some workers have had poor results with needles and prefer to tie a cannula in a vein. This technique has the disadvantage that when the cannula is removed the vein must be tied and cannot be used again. In any case the vein usually becomes inflamed after a few days and the site of injection must then be changed. The number of available veins is large but not infinite.

The fluid should contain isotonic sodium chloride (1%) or glucose (5%). Various workers have proposed that heparin should be added to prevent thrombosis (Martin, 1944). A common rate of injection is 2·5 cm.³ per minute or 3·6 l. in 24 hours. The apparatus is usually a simple reservoir 2 feet [0·6 m.] above the patient, and the rate is controlled by a screw-clip and a drip-feed.

Marrow Infusions

The administration of fluids into the bone-marrow was first described by Tocantins & O'Neill (1941). Such fluids pass directly into the blood-stream, and even blood itself can be given in this way. The rate may be as high as 25 cm.³ per minute, and this method has been recommended as easy, quick and safe when veins are difficult to find (Bailey, 1944; Morgan, Christie & Roxburgh, 1944). The infusion of penicillin into the marrow near the site of osteomyelitis should be particularly effective by producing a high local concentration as well as an adequate concentration in the systemic blood (Aird, 1945).

Subcutaneous and Intramuscular Injections

These have the advantage of simplicity; the needle can be inserted by a nurse. Fluids may be given either continuously or in repeated doses. For continuous administration the intramuscular method is best, since absorption is quicker. Using two needles in the thigh muscles, fluids can be given as rapidly as 5 litres in 24 hours, or 3·5 cm.³ per minute (Billimoria & Dunlop, 1940), but high rates are liable to cause pain, and it is generally agreed that the volume given by this route in 24 hours should be as small as possible. The slower the rate of injection the more difficult it is to get a steady flow, and various devices have been used for this purpose. One simple apparatus which delivers 100 cm.³ in 24 hours is known as Eudrip III (McAdam, Duguid & Challinor, 1944); the rate is controlled by the flow of air through a capillary tube. Various authors have described electrically-operated machires which gradually push in the piston of a syringe. In this way the rate may be reduced to as low as 2 cm.³ per 24 hours. The need for economy in the use of penicillin has been a potent stimulus to the invention of such methods of administration. Now that penicillin is becoming more plentiful, there is a tendency to revert to the simpler procedure of repeated injections.

When repeated injections are used it is usually an advantage if the drug is slowly absorbed so that a fairly steady concentration is maintained in the blood. There are many methods of forming such depots of drug, and some of them are apt to be too effective, so that an adequate concentration in the blood is never reached. The route of absorption depends on the molecular weight of the substance absorbed. When this is greater than 20,000 (which is about that of the smallest protein molecules), absorption is by lymphatics and can be slowed by immobilizing the site of injection. Smaller molecules are absorbed by the blood (Barnes & Trueta, 1941).

The rate of absorption depends on a number of factors:

i. *Site of injection:* Intramuscular injections are usually absorbed more quickly than subcutaneous injections.

ii. *Local circulation:* Warmth dilates the blood-vessels and quickens absorption, and cold has the opposite effects. Trumper & Hutter (1944) have recommended that the site of injection of penicillin should be chilled to slow absorption and diminish pain. The circulation can also be slowed by adding a vasoconstrictor such as adrenaline to the injection, and this method is used to delay the absorption of local anaesthetics.

iii. *Surface-area of the depot:* A watery solution of a bismuth salt is absorbed more rapidly than an oily solution, partly because it is less viscous and spreads more widely in the tissues. The effect of the surface-area is also shown particularly clearly when pellets of steroid hormones are implanted subcutaneously. Such pellets may continue to be absorbed for many months (Thorn, Dorrance & Day, 1942; Dunlop, 1943). They can be taken out at intervals and weighed and measured. In this way it has been found that the rate of absorption is directly proportional to the surface-area (Bishop & Folley, 1944).

iv. *Solubility of the drug and its vehicle in the tissue-fluids:* This is the most important factor, and there are many ways of taking advantage of it. The solubility of the drug can be controlled by converting it into a salt with the appropriate properties. Experiments with steroid hormones have shown that this has a marked effect on the rate of absorption. The solubility of the vehicle can also be controlled. Drugs may be given suspended or dissolved in water or in fatty oil. Protamine-insulin is a relatively insoluble preparation which is injected in watery suspension. Globin insulin and bismuth tartrate are injected in watery solution, but are immediately precipitated in the tissues and then slowly dissolved. Propylene glycol can be used in much the same way. It is readily miscible with water, but dissolves steroid hormones and other fat-soluble substances. When such solutions are injected the solvent mixes with the tissue-fluids and the drug is precipitated and then slowly dissolved. Water-soluble substances such as adrenaline (Kennedy, 1941) or pitressin tannate (Court & Taylor, 1943) or sodium penicillin (Raiziss, 1944) are slowly absorbed when injected in suspension in oil, but oils are more commonly used for drugs which dissolve in them.

When liquid fats such as peanut (arachis) oil or sesame oil, or esters such as ethyl oleate, are injected intramuscularly, they and the drug are slowly absorbed. The rates of absorption and local irritant effects of various oils have been studied by Brown, Wilder & Schwartz (1944), who came to the conclusion that sesame oil and maize oil were preferable to cottonseed oil and peanut oil. The rate of absorption of these oils can be slowed by adding substances such as beeswax, which consists of long-chain alcohols and fatty acids and their esters. It is relatively insoluble in water and diminishes the solubility of the oil. It has been used to delay the absorption of histamine, desoxycorticosterone acetate, heparin, and penicillin (Romansky & Rittman, 1944).

Respiratory Tract

The mucous membrane of the nose is a fairly effective absorbing surface, and snuffs containing nicotine, cocaine or heroin give satisfaction when applied to it. Pituitary extracts have been given by this route, but the method is not very reliable. Drugs applied to the nose are usually intended to have local effects, but if large quantities of oil are given in this way as a solvent, it may run down into the lungs and cause pneumonia (Robertson, 1940).

Sprays are sometimes used to administer drugs to the lungs. The simplest forms of spray produce large drops which are deposited in the nose. It is only droplets with a diameter of 5 μ or less which reach the lungs, and these form a stable cloud which shows no tendency to settle. Such clouds are produced by sprays containing a baffle, which returns all but the smallest drops to the input of the spray (Collison, 1935). Water-soluble substances which reach the lungs are very rapidly absorbed into the general circulation, and a lung may absorb several times its own weight of water in an hour. On the other hand, it is difficult to give much drug in this way. The total weight of the drops in a cloud is commonly about 5-10 mg. per litre. If, therefore, it is possible to use a 10% solution of the drug and to induce the patient to inhale 10 litres per minute and retain all the drops, he will receive about 5-10 mg. of drug per minute. Usually the rate of administration will be less than this, and prolonged inhalation will be necessary to get much drug into the systemic circulation.

On the other hand, such sprays produce a high concentration of the drug on the walls of the bronchi, and it is not difficult to produce effective concentrations of drugs in the sputum. This method of administration is thus most likely to be effective in the treatment of infections of the bronchi. Both sulphonamides (Mutch, 1944) and penicillin (Bryson, Sansome & Laskin, 1944) can be used in this way.

Serous Cavities

The permeability of the linings of the body-cavities is important in the treatment of infections of these cavities. The sulphonamides usually penetrate serous membranes easily, but penicillin normally does not do so and is often given by local injection. When the membrane is inflamed its permeability in both directions is increased. Systemic administration is therefore likely to be more effective in the treatment of infections of the pleura, meninges and joints than might be expected from experiments on normal subjects. The peritoneum is more permeable than other membranes. Penicillin is given by injection into the pleural cavity, joints, and cerebrospinal fluid in the treatment of local infections of these parts, and an effective concentration is usually obtained for much longer than would have been the case if the same dose had been given systemically. It must be remembered, however, that such infections usually involve the tissues surrounding the cavity as well as the fluid inside, and it is often desirable, therefore, to maintain an effective concentration in the blood as well as in the cavity. When sufficient penicillin is injected locally in such cases, it may actually increase the blood-concentration effectively for long periods through slow absorption of the drug. It has even been suggested that penicillin should be injected into normal cavities in order to treat infections elsewhere, but the rate of absorption is not likely to be under good control in these circumstances, and it is doubtful whether this method of forming a depot will have wide application.

Wounds and Burns

The application of drugs to wounds has been discussed by Robson (1943) and Hawking (1943). In parts of a wound where the circulation is good, the local administration of drugs is unlikely to be effective because the drug is rapidly absorbed, and systemic administration is more likely to achieve results. Where the circulation is bad, the only effective way of using drugs is to apply them locally. Drugs diffuse very slowly over distances of 1 cm. or more unless they are carried by convection currents, and it is therefore necessary to ensure that the method of application carries the drug to all parts of the wound. Dead tissues form a barrier to drugs, and their removal is therefore desirable for this reason as well as others.

Drugs are liable to disappear rapidly from wounds, either because they are absorbed or because they are inactivated. It is therefore usually necessary to apply the drug continuously or repeatedly, or to form a local depot from which the drug is slowly liberated. The continuous irrigation of wounds has been much used, and is particularly likely to be effective when the wound is enclosed in a bag through which a solution of the drug runs. Penicillin is effective when given intermittently through a rubber tube inserted through the skin when a wound is sewn up. Local depots can be formed by the use of powders which are sprinkled or insufflated over the wound and as far as possible into crevices. The concentration of the drug in the wound then depends on its solubility. Sulphanilamide is fairly soluble and effective. Its rate of absorption is very roughly half its rate of absorption from the intestine (Hawking, 1943), and it may therefore cause general toxic effects if excessive quantities are used. Many other sulphonamides are relatively insoluble, and are effective only when a very fine powder is used.

Drugs may also be applied as creams particularly in the treatment of burns (Robson & Wallace, 1941; Colebrook, Clark, Gibson & Todd, 1944). The use of sprays is convenient, but drugs applied in watery solution in sprays are liable to be quickly absorbed. When the drug is incorporated in dressings it is not likely to reach deeper parts of wounds.

Eyes

Drugs do not pass readily from the blood-stream into those ocular tissues which have a poor blood-supply; they are therefore often applied locally. The application of a 30% solution of sodium sulphacetamide to the surface of the conjunctiva is effective in the treatment of corneal ulcers (Robson & Scott, 1941). This particular sulphonamide was chosen because it is very soluble at neutral *p*H. Penicillin is also effective when used in the same way. Under suitable conditions, drugs penetrate to the cornea, sclera, aqueous humour and iris. The main barrier to penetration is the epithelium on the surface of the cornea (Ginsburg & Robson, 1945). The penetration of local anaesthetics through this epithelium is increased if the solution is alkaline, since the free base penetrates more readily than ions. Wetting-agents also increase the penetration of sulphonamides, but not of penicillin, by destroying the epithelium. When given in drops, the drug disappears rapidly, and hourly administration is recommended. The labour involved in such frequent application can be avoided to some extent by the use of cotton packs soaked in the drug or a corneal bath. A more effective method is to inject the drug subconjunctivally or into the aqueous, or vitreous, humour (Brown, 1946).

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[1] *[See BMB* 719/127]
[2] *[See BMB* 318]

History

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The important contributions of France to chemotherapy have received relatively little attention in some of the relevant literature. The article below was contributed by invitation, and is a first-hand account of the chemotherapeutic work of the Institut Pasteur. It has been placed for convenience in our Historical Section, although the Institut Pasteur is essentially a part of the medical history, not of the past, but of our own time.

THE CONTRIBUTION OF THE INSTITUT PASTEUR, PARIS, TO RECENT ADVANCES IN MICROBIAL AND FUNCTIONAL CHEMOTHERAPY *

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Protozoa and Spirochaetes

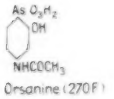
i. *Trypanosomiasis*

At the Institut Pasteur in 1902, Laveran and Mesnil[1] established the conditions necessary for transmitting trypanosomiasis from one animal to another. Mesnil and Nicolle[2] in their later studies of this disease demonstrated the therapeutic properties of certain dyes, such as afridol violet and trypan blue, and also of several arsenical compounds.

The study of the latter group of compounds was continued by Ernest Fourneau, who is justly considered to be the successor of Ehrlich, the pioneer of chemotherapy. Fourneau, appointed director of the Department of Therapeutic Chemistry at the Institut Pasteur in 1911, contributed to the identification of atoxyl, the first active drug that could be used with safety in the treatment of human and animal trypanosomiasis.

The exact determination of the structural formula of atoxyl has had a decisive effect on further research on the arsenical drugs: once it was known that atoxyl was not an arsenical salt of aniline but that the arsenic was directly attached to the benzene ring, it was possible to change the position of the amino group, or the group itself could be altered, replaced or supplemented by other groups. It is the study of derivatives obtained in this manner which we have pursued, especially since 1921, in the Laboratory of Therapeutic Chemistry, and which led us to the demonstration of the chemotherapeutic properties of orsanine and stovarsol[3].

FIG. 1



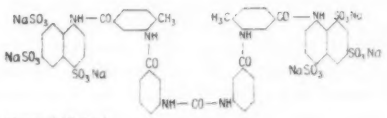
Orsanine (270F)

Orsanine (Fourneau 270) (Fig. 1) is the remedy for the first stage and the beginning of the second stage of sleeping sickness, as the American tryparsamide is for the final stage of this disease. Moreover, treatment with a combination of orsanine and moranyl is an example of true synergy, since the latter re-sensitizes trypanosomes which have become resistant to the action of arsenical drugs. The history of moranyl (Fourneau 309) (Fig. 2) is worth recording: the formula of this substance is identical with that of germanin (Bayer 205),

* *Translated from the French by A. H.-S, with assistance from Dr. F. R. Selbie*

which the Germans refused to disclose when tests were being carried out in Africa. Bayer 205 has an exceptionally high therapeutic index in mice, the toxic dose being more than 300 times greater than the therapeutically active dose. The first results in man were so promising that the Germans

FIG. 2



Moranyl (309 F)

offered to hand over the formula of Bayer 205 in exchange for the Cameroons. Fourneau, Tréfouël, Tréfouël and Vallée[4], however, were fortunate enough to discover the formula of this substance and published it at once. They also demonstrated the amazing specificity of its trypanocidal properties : in fact, by simply removing the CH_3 group or transferring it from the first to the second benzene ring, it was possible to cause a decrease, or even complete disappearance, of activity.

Fourneau, Tréfouël, Tréfouël, Bovet and Koetschet[5] showed later that the polyarsenical derivatives have a selective action on *Trypanosoma congolense*, whereas the monoarsenical derivatives are totally inactive.

Before leaving the field of trypanosomiasis, mention should be made of the work of Funke and his co-workers[6], who demonstrated the trypanocidal properties of Fourneau

FIG. 3

1921 F

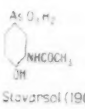
1921 (Fig. 3), the most active member of a series of diamines that had no relationship to any of the previously known series of trypanocides. Fourneau 1921 is active whether it is given subcutaneously or orally.

ii. *Spirochaetal infections*

The activity of bismuth in syphilis was first discovered at the Institut Pasteur by Sazerac and Levaditi in 1922.[7] Bismuth still plays an important part in the therapy of this disease along with mercury, arsenic and penicillin.

In the course of our study of the arsenical acids we found in stovarsol (Fourneau 190) (Fig. 4) another striking example of

FIG. 4



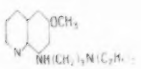
Stovarsol (190 F)

the interdependence of therapeutic properties and chemical constitution. Examination of its formula shows that it differs from orsanine only in the position of the groups $NHCOCH_3$ and OH in relation to the arsenic acid group. And yet, while orsanine is the specific for sleeping sickness, stovarsol is the specific for the spirochaetal infections, syphilis and yaws.[8,9] It is active by mouth and gives excellent results in natural and experimental tertiary syphilis. It is also active against certain intestinal parasites (*Giardia lamblia*, amoebae[10], *Blastocystis hominis*) and *Plasmodium vivax*[11].

iii. *Malaria*

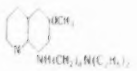
About 1928 Fourneau's team opened a new research laboratory for the study of synthetic antimalarial drugs in the canary and Java sparrow [*Padda oryzivora*]. In the series of quinolines with a carbon-nitrogen side-chain we have found some interesting relationships between chemical constitution and activity against various forms of plasmodia[12] : in particular, we have shown that with progressive lengthening of the carbon side-chain there is a diminution of activity on gametes, whereas the effect on schizonts is non-existent with the shorter carbon chains and gradually increases, as the side-chain is lengthened, to become quite definite with the group C_{11} (Fourneau 852) (Fig. 5, 6) of which the stovarsol salt is Fourneau 915.

FIG. 5



Rhodoquine (710 F)

FIG. 6

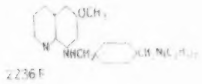


852 F

This represents the logical link uniting the series of plasmoquin, the gametocide, to that of quinine, the schizonticide. The first clinical tests were carried out by Marchoux and Chorine [13].

Funke, Bovet and Montézin[14] have studied the effect of interpolating an aromatic nucleus in the carbon chain,

FIG. 7



2236 F

Among the substances tested, Fourneau 2236 (Fig. 7) showed a characteristic antimalarial activity against experimental avian infection (canaries, fowls). Its relatively low toxicity for animals justifies its clinical trial.

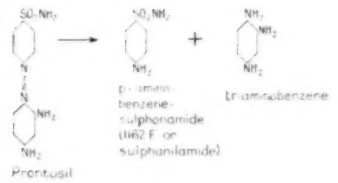
Bacteria and Filtrable Viruses

i. *Sulphonamides*

The development of antibacterial chemotherapy dates from 1935, when Domagk[15] demonstrated the antistreptococcal properties of prontosil. We immediately commenced a study of prontosil derivatives, applying the chemical and biological principles that had proved their value in antiprotozoal chemotherapy. In a very short time we discovered that the usual rule of specificity of action was no longer valid for this series, at least in so far as changes in the second nucleus of prontosil were concerned, since none of these affected antibacterial activity. We thought it logical to assume that this second nucleus played only a secondary therapeutic role, if any ; on the other hand, knowing that the azo linkage is relatively weak, we put forward the conception that prontosil, under the influence of constant oxidation-reduction reactions in the body, would be split at the link $-N=N-$ by the acquisition of hydrogen and the

liberation of two independent molecules, that of *p*-aminobenzenesulphonamide from the first nucleus and that of triaminobenzene from the second nucleus of prontosil (Fig. 8).

FIG. 8

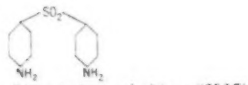


As this second nucleus did not seem to play any part in therapeutic activity, we prepared *p*-aminobenzenesulphonamide (Fourneau 1162 or sulphanilamide), which Gelmo had prepared as early as 1908, and carried out tests with it on mice. The first results were conclusive, showing that streptococcal infection in mice was no more resistant to sulphanilamide than to prontosil and was, in fact, more sensitive to the new drug[16]. This observation solved the apparent paradox of the complete inactivity of prontosil *in vitro* compared with its activity *in vivo*. In fact, prontosil cannot act *in vitro* since it retains its identity ; it becomes active only when it is transformed by the animal organism into sulphanilamide, which is active both *in vitro* and *in vivo*. From then onwards, the study of the azo-derivatives was completely abandoned, and sulphanilamide became the starting-point for further research by bacteriologists and chemists.

We then showed[17] that sulphur could manifest its activity in other forms than that of the sulphonamido group $-SO_2NH_2$. Activity increases with the degree of sulphur oxidation, and we have been able to classify sulphur-derivatives in order of interest as follows : sulphides, sulphoxides, sulphones.

Of all the substances we have tested, di(*p*-aminobenzene) sulphone (Fourneau 1358) (Fig. 9) is the most active against

FIG. 9



streptococcal and pneumococcal infections in the mouse. Our friend Dr. Buttle reached an identical conclusion at the same time as we did. But Fourneau 1358 is extremely toxic and shows no evidence of the exceptionally wide range of activity displayed by sulphanilamide. Rodilone (Fourneau 1399) can be obtained by means of diacetylation.

By means of experiments on *Aspergillus niger* we were able in 1936 to outline the mode of action of sulphanilamide[18] and to conclude that it does not exert an antiseptic action, but simply one of bacteriostatic inhibition. It " suspends life ": the recent experiments of Lwoff and others[19] on a flagellate of fairly large size, *Polytomella caeca*, allow us to follow this mode of action with ease : after two or three normal divisions, flagellates of considerably increased size are obtained ; these are unable to multiply, but their vitality is completely preserved, since they again divide normally after the addition of an antisulphonamide, such as *p*-aminobenzoic acid. These experiments have, moreover, shown that the relative activities of sulphanilamide and *p*-aminobenzoic acid are determined by cell-permeability, the maximum bacteriostatic activity being at *p*H 7, and the maximum antisulphonamide activity being at the isoelectric point of *p*H 3·8.

Nitti and others[20] have investigated the production of antisulphonamides in culture media : they confirmed that antisulphonamide activity accumulates in the most degraded protein fractions, and tested the activity of some of the amino-acids that constitute the final products of the protein degradation scale : two of these, *dl*-leucine and *dl*-methionine manifested some activity, but this was inferior to that of *p*-aminobenzoic acid. The same authors have also synthesized a mixture closely resembling a final hydrolysate of myosin and have been able to show that this mixture, which is inactive by itself, has a definitely stimulating effect on the activity of *p*-aminobenzoic acid ; possibly this results from an increase in the ability of the bacteria to produce *p*-aminobenzoic acid. Finally, these authors have shown that the comparative antisulphonamide activity of peptones and of *p*-aminobenzoic acid varies with the nature of the bacteria : cultures of *Proteus*, *B. coli*, and Friedländer's bacillus that are inhibited by sulphanilamide resume growth equally well with the addition of either *p*-aminobenzoic acid or peptone, whereas with cultures of streptococci and pneumococci growth is re-stimulated by *p*-aminobenzoic acid but not by peptone.

Our knowledge of the antisulphonamide factors in peptone media led to a better understanding of the local use of sulphonamides in the treatment of wounds : by showing that sulphanilamide inhibits the development of streptococci and *Cl. welchii*, which were together responsible for 75% of the deaths in the 1914-18 war, the experimental investigations of Legroux and Nitti[21] on guinea-pigs and rabbits made possible the treatment of the war-wounded in the spring of 1940.

The sensitivity of a micro-organism to sulphonamides depends not only on its *nature*, but also on its *virulence*. This was shown by Nitti and Bovet[22] : microbes of low virulence give rise to infections which are much more difficult to suppress ; on the other hand, when microbes suspended in mucin are injected, their virulence is increased and chemotherapy once again becomes effective.

Another factor which influences the action of drugs is the development of resistance to chemotherapy in certain micro-organisms. Roux and Chevé[23] have shown that strains of meningococci can be trained to withstand increasing concentrations of sulphanilamide and sulphapyridine. But, whereas in the first case virulence is reduced, the strains rendered resistant to sulphapyridine have retained their virulence and can cause infections in mice that are refractory to treatment not only with sulphapyridine but also with sulphanilamide.

In order to render sulphanilamide active against the staphylococcus in cases of furunculosis or carbuncle in which vascularization is deficient, Legroux had the idea of combining sulphonamide treatment with the resolvent properties of iodine ; iodine softens the nodular lesions and thus allows sulphanilamide to exert its action on the originally encysted staphylococci.[24]

The first clinical experiments with Fourneau 1162 (sulphanilamide) were carried out at the Hôpital de l'Institut Pasteur by Martin and Delaunay[25]. In the course of the following years, Martin and his co-workers[26] established the principles of dosage and methods of estimation which made the use of sulphonamides possible in France with the maximum safety and efficiency.

ii. *Penicillin*

It was this same team that was the first in France to experiment with penicillin during the occupation years. Thanks to the combined efforts of the Rhône-Poulenc Company and F. Nitti, a small quantity of penicillin was prepared and the first cases treated with this drug date from 1944[27]. Later, our hospital was among the first to receive penicillin from England and America, and this made it possible to extend the original results[28].

In order to increase our stock still further, we established in March 1945 a laboratory for the recovery of penicillin from the urine of patients undergoing treatment[29].

The laboratory for the estimation of penicillin added to previously known techniques by perfecting A. Prévot's method[30], which was based on an entirely new principle: the test organism, *Cl. welchii* or *Cl. butyricum*, decolorizes fanus green by means of reduction in about an hour, and if the amount of penicillin present is sufficient to inhibit the growth of these bacteria, there is no decolorization. The method is sensitive and rapid, giving results in about 2 hours.

We have already said that Nitti was in possession of penicillin in 1944. With the aid of the microbiophotometer, which was constructed by Faguet and Nitti[31], and registers bacterial growth-curves, it was possible to demonstrate the mode of action of penicillin: in the first stage, lasting 2-3 hours, growth is slowed down, then there is progressive lysis, which becomes complete in a few hours if the quantity of penicillin is sufficient. Lysis is at its maximum when growth is most active. Lafaille, Sureau and Berrod[32] have shown that the toxicity of penicillin to cultures of diphtheria bacilli is inversely proportional to the age of the culture.

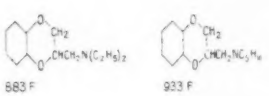
As it was not generally accepted that there is a synergy of action between penicillin and sulphonamides, this subject was reinvestigated at the Institut Pasteur both experimentally and clinically. Nitti, Boyer and Faguet read a communication[33] to the Association des Microbiologistes de Langue Française in January, 1946, in which they showed the undoubted value of combining these two drugs in experimental streptococcal or pneumococcal infection in mice. This synergy is logical, as the two drugs act in different ways. The experimental findings *in vivo* have been confirmed both by tests *in vitro* and by clinical experience.

These conclusions are fully supported in the excellent book by Martin and his collaborators[34], and in Simonnet's memoir[35].

Treatment of Functional Disorders

The origin of the investigations undertaken in the Laboratories of Therapeutic Chemistry on poisons of the autonomic nervous system is to be found in the partly accidental observation that adrenalin is antagonized by the aminomethylbenzodioxan derivatives, Fourneau 883 and Fourneau 933 (Fig. 10), which were first synthesized in connexion with

FIG. 10

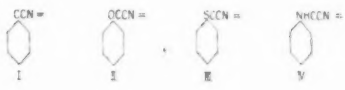


our research programme for the discovery of antimalarial drugs. In these derivatives, sympathicolytic action is found at its maximum; paralysis, or reversal, of the effects of adrenalin appears after a very small dose and affects a very great number of organs and functions: blood-pressure, capillaries, heart, iris, nictitating membrane, unstriped muscles of the intestine and uterus, bronchi and melanophores[36].

The fact that the physiological activity of the aminomethylbenzodioxans is connected with the exact correspondence of the molecule and some cellular structure stands out with particular clarity from the work which has been done on the stereo-isomers. The ratio between the pharmacodynamic activity of the left and right isomers[37] is 6 : 1 and in some cases 20 : 1.

Later we turned our attention to the problem of the mode of action of these substances and in particular to the structural relationships which would permit the comparison of adrenalin and the sympathicomimetics with their antagonists. Apart from the well-known series of the benzylethylamines (I), we discovered the sympathicomimetic activity of several series of new derivatives, the phenoxyethylamines (II), the benzylethylsulphides (III) and the benzylethylenediamines (IV)* (Fig. 11), each of these new series being, moreover, an

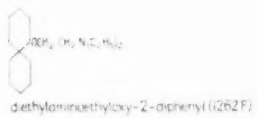
FIG. 11



intermediary stage between what are usually called the sympathetic "stimulants" and their antagonists, the sympathetic "paralyzers". We have described new synthetic sympathicolytics in this way in the following chemical series: phenoxyethylamines[39], aminomethylcoumarins[40], benzylethylsulphides[41], benzylethanolhydrazines[42], aminobenzylmorpholines[43] and benzylethylenediamines[44].

As the result of a suggestion that we should extend the field of investigations begun by our researches on the phenol esters and the aminomethylbenzodioxans, we were led to study the antagonism shown by a whole series of new diphenyl esters to fibrillations caused by physical or chemical agents. The systematic study of these showed that the most active group of this series was diethylaminoethyloxy-2-diphenyl or Fourneau 1262[45] (Fig. 12). This product is now used in the treatment of angina pectoris.

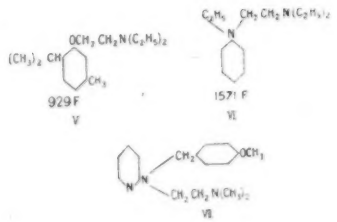
FIG. 12



The pharmacological study which grew out of the investigation of the phenol esters also led us to the conception of antihistamine poisons. It is well known that the biologically highly active amines, acetylcholine, adrenalin and histamine, are related in their chemical structure, distribution in body fluids and pharmacodynamic properties. It thus appeared likely that, just as there are alkaloids capable of opposing the effects of acetylcholine and sympathicolytic poisons which neutralize or reverse the effects of adrenalin, there might be substances possessed of a specific antagonism to histamine. These synthetic antihistamine compounds were discovered, in our laboratory, in three chemical series derived respectively from phenol (V), aniline (VI), and aminopyridine

(VII) (Fig. 13). N - *p* - methoxybenzyl - N - dimethylamino-ethylaminopyridine (VII) neutralizes the toxic effect on the guinea-pig of 100 toxic doses of histamine, and acts with a dose as low as 0.1 mg. per kg. Both in France and abroad

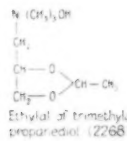
FIG. 13



extensive work has established as classic the conception of the specificity of the action of the histamine antagonists and the explanation of their action by postulating a blocking of the specific peripheral receptors. Experiments carried out with products of this type provide an additional proof of the part played by histamine in anaphylactic shock. The antiallergic action is clearly apparent in anaphylactic shock in guinea-pigs, rabbits and dogs, in the Sanarelli-Shwartzman reaction and in the Schutz-Dale phenomenon. Various products from these series are now used clinically in serum sickness, urticaria, Quincke's oedema, and various forms of asthma[46 47].

A recently obtained member of the group of acetylcholine derivatives—ethylal of trimethylammonium propanediol (Fourneau 2268) (Fig. 14)—probably represents the most

FIG. 14



active parasympathicomimetic derivative known, being toxic in doses of the order of 0.001 mg. per kg. A similar compound is at present being studied clinically[48].

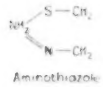
Particular mention must also be made of a new group of nitric esters of amino-alcohols, with a remarkably simple structure (Fourneau 2054) (Fig. 15) and with an action closely

FIG. 15

allied to that of the nitrites and of trinitrin ; Fourneau 2054 is just as efficacious as the latter product, but is much less toxic[49].

In a series of publications we have described the experimental and clinical findings obtained with aminothiazole (Fig. 16) in counteracting thyroid function and in its use for

FIG. 16



the correction of hyperthyroidism. It may be concluded from the clinical researches carried out in France, that aminothiazole, by reason of its almost complete innocuity, appears to take pride of place over other substances previously suggested, such as thiourea and thiouracil[50]. The experimental work has been based on both pathological and physiological studies. By means of a technique which makes use of radioactive iodine, it has been possible, in tests carried out in collaboration with Joliot-Curie, to show that, in the living animal, aminothiazole antagonizes the synthesis of thyroxine[51].

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CLAUDE BERNARD AND SCIENTIFIC ADVENTURE

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The importance of Claude Bernard as one of the chief founders of modern experimental physiology was recognized even during his lifetime and his fame has grown with the passing years. A reviewer's statement that all recent French philosophy of science takes its origin in Claude Bernard[1] may be an exaggeration, but at least it testifies to the growing realization of his significance as a scientific philosopher. Within the last 10 years a number of his philosophical writings have appeared in print for the first time, and his *Principes de la médecine*, which was still unfinished at his death, now awaits publication. J. M. D. Olmsted's detailed and scholarly biography[2], which appeared in 1939, is now followed by a more popular exposition of Bernard's life and work by Raymond Millet, entitled *Claude Bernard ou l'aventure scientifique*[3].

It would be difficult to achieve for Bernard what Vallery-Radot so nobly accomplished for Bernard's fellow-countryman and contemporary, Louis Pasteur, and it would be almost impossible to find in Bernard's life and work the popular appeal to be derived from many writings on men such as Paul Ehrlich ; yet M. Millet has almost achieved the impossible. Although Michael Foster's biography of Claude Bernard, published in 1899[4], describes the scientific career of the great Frenchman, it makes little attempt to depict him as a human being, and indeed the picture of his life as a whole began to emerge only with the publication of Olmsted's masterly study. The development of the portrait thus begun is well carried forward by M. Millet's latest work.

The outlines of Bernard's life are easily told. He was born in 1813 at Saint-Julien-en-Beaujolais of peasant stock. While serving as assistant to a pharmacist in Lyons at the age of 20 he wrote and had professionally produced locally a comedy sketch entitled *La Rose du Rhône*. Thus encouraged, he gave up pharmacy for the composition of a full-blooded five-act drama, *Arthur de Bretagne*, which, in 1834, at the age of 21, he took to Paris. There he was advised by M. Girardin, the critic, to study medicine rather than the drama, advice which he followed, albeit somewhat crestfallen. Qualification in medicine was quickly followed by appointment as assistant to Magendie, then professor of medicine at the Collège de France, whom Bernard later succeeded. His discovery that the liver secretes sugar (*Nouvelle fonction du foie*, 1850, 1853)[5] and his isolation of glycogen (1857) quickly established his fame as an investigator of first rank.

It is no exaggeration to say that during his active years there was no field of experimental physiology which did not receive some attention from Bernard, and to almost every subject that he submitted to investigation he made some substantial contribution. But scientific fame accompanied domestic unhappiness, culminating in formal separation from his wife and daughters in 1870. His wife had from the first been unsympathetic and even antipathetic to her husband's investigations, involving as they did vivisection, and it must be confessed that in some ways Bernard was not a lovable personality. His own account of how he took a sick dog home for the week-end in order to observe closely the development of the animal's illness may well give a clue to the background of his domestic unhappiness. About the time of the legal separation from his wife, there developed an apparently platonic friendship between Bernard and

Mme. Marie Raffalovich, a friendship which lasted until his death in 1878. There are still preserved in Paris some 500 unpublished manuscript letters from Bernard to Mme. Raffalovich written between 1869 and 1878. The light these throw on Bernard's private life at this time is of unique importance.

To the man of science, the development of Claude Bernard's researches holds perhaps more interest than the revelation of his domestic affairs, or the assessment of his worth as a scientific philosopher. Like other classics, many of Bernard's writings are seldom read; nevertheless, their putative contents are often handed down from textbook to textbook like a precious heirloom of alleged but unascertained worth. His views on the question of whether or not liver-glycogen can act as a storage-form of administered glucose are usually mis-stated, and examination of his writings in some detail shows Bernard's own uncertainty on this point[6]. Likewise his views on the glycogenic function of the liver are sometimes much more lucidly expressed in textbooks than in Bernard's own writings.

Some of his earlier publications can give courage to those struggling with their own difficulties by demonstrating the enormity of some early mistakes of the great physiologist. There can be little doubt that the results of his investigations on secretory nerves coloured, and coloured in false tones, all his early ideas on the secretion of sugar by the liver, and his early belief that section of the vagus supplying the liver causes the disappearance of sugar from that organ[7] arose naturally from a preconceived idea that the vagus must be the secretory nerve concerned. It was such preconceived but incorrect notions that led to the discovery of piqûre "diabetes", and to the interesting theory that the stimulus to the secretion of sugar by the liver was initiated in the lungs and carried by the vagus to a nervous centre in the medulla, whence it reached the liver by way of the spinal cord and splanchnic nerves[8].

It is clear, too, that many of Bernard's early experiments on carbohydrate metabolism were carried out under such unphysiological conditions as to yield results quite inapplicable to the normal animal. In this respect F. W. Pavy's criticisms[9] of Bernard's early experiments were well justified. As a young English medical graduate, Pavy had visited Bernard's laboratory in 1854 and had seen repetitions of the experiment whereby Bernard had demonstrated that the liver of the starving or meat-fed dog secretes large amounts of sugar. In this experiment section of the medulla was first carried out in order to arrest life "in an essentially normal state"; the blood of the hepatic veins was then demonstrated to contain an abundance of sugar while the blood of the portal veins contained none. On his return to England, Pavy decided to investigate the disappearance of the sugar from the blood when it was perfused through surviving lungs, and for this purpose he withdrew blood from the right heart of the unanaesthetized living dog by catheterization of the jugular vein. To his surprise he found that blood obtained in this way contained little or no sugar unless the animal struggled or became asphyxiated. When however, an animal was killed, the right heart was found to contain an abundance of sugar. As the result of these and many other experiments, Pavy concluded that Bernard's glycogenic function of the liver was a pathological phenomenon of no relevance to the living normal animal. Finding later that the sugar content of the portal-vein blood was significantly higher than that of the hepatic veins for some time after a dog had ingested a carbohydrate meal, Pavy concluded that the liver was an organ which absorbed rather than secreted sugar, converting the absorbed glucose into glycogen[9]. This was in direct opposition to Bernard's ideas and a controversy developed which was still unresolved at the time of Bernard's death[6]. To the end of his long life (he died in 1911), Pavy maintained that the production of sugar by the liver was a phenomenon of pathological but not of physiological interest, despite the ever-increasing body of evidence in support of Bernard's view that the secretion of sugar by the liver was an essential factor in the economy of the normal animal.

Although his work is now largely forgotten, it is clear that many of Pavy's criticisms of Bernard's work were justified. In his early experiments, carried out on a pithed or dying animal, Bernard must have stimulated hepatic glycogenolysis to a substantial extent, so that the sugar content of the blood of the hepatic veins became quite abnormally high. In his early investigations Bernard was unable to find any sugar at all in the portal-vein blood of the starving dog, and it seems very probable that if his experimental conditions had not resulted in the stimulation of hepatic glycogenolysis, he would have missed the sugar in the blood of the hepatic veins as well as that in the blood of the portal vein, and would thus have failed to discover the glycogenic function of the liver and would not have isolated glycogen. Pavy, on the other hand, was able to detect the sugar in portal-vein blood in his earliest experiments but, because his carefully-chosen conditions prevented post-mortem hepatic glycogenolysis, he concluded that the blood in the hepatic veins contained the amount of sugar which was normally indistinguishable from that of the blood in portal veins.

In his experimental facts, Pavy was therefore far nearer the truth than was Bernard at that time. Hence, the curious paradox arises that, if Bernard had chosen more-physiological conditions for his early experiments, he might never have discovered glycogen and the glycogenic function of the liver, while if Pavy had been a little less rigid in his determination to use strictly physiological conditions, he might not have been led to develop his incorrect theories of carbohydrate metabolism. Undoubtedly Bernard's strength lay in his ability to discard ruthlessly an incorrect theory once he was led to conclude that its experimental bases were at fault, an ability which Pavy did not share to such a marked degree. Nevertheless, the fact that Claude Bernard, a man of Olympian aloofness of intellect, could at times make such egregious mistakes, may bring some degree of comfort and consolation to us all.

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The Library

Publications discussed or listed in this section may be borrowed by inquirers resident in the United Kingdom on application to the Editor.

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PHYSIOLOGY OF THE HUMAN EYE

A. Magitot: *Physiologie oculaire clinique.* Masson, Paris, 1946. 750 francs.

Though ophthalmology is rich in monograph literature on the comparative physiology of the eye, there are remarkably few systematic expositions on the physiology of the human eye, possibly because the subject is so fluid. The publication of *Physiologie oculaire clinique*, by A. Magitot, is therefore particularly welcome. The author has himself contributed extensively to the subject, particularly on the physiology of the intraocular fluids, and this book brings together critically and exhaustively a mass of scattered information on ocular physiology generally. The field covered is extensive, as is seen from the chapter-headings. There are fifteen sections, several of them containing subdivisions: i. Protective mechanism of the globe. ii. Ocular sensitivity. iii. Nutrition of the globe. iv. Intraocular fluids. v. Ocular tension. vi. Circulation of the conjunctiva. vii. Cornea. viii. Iris and pupil. ix. Lens. x. The retina. xi. The function of the retina. xii. The optic nerve. xiii. The visual cortex. xiv. Ocular muscles and ocular motility. xv. Binocular vision.

This treatise has Gallic elegance and clarity—to use an overworked but thoroughly applicable phrase. It is extensively illustrated and the presentation leaves little to be desired. The bibliographies at the end of each chapter, whilst not exhaustive, are adequate.

The book is intended for the clinician. It lacks any discussion of the refractive state, and contains but little on physiological optics, but these subjects are adequately covered by existing monographs. Magitot's treatise is particularly good in the stress it lays on general physiology in the understanding of the physiology of the eye, and on the significance of the study of pathological states in the problems of physiology. It should do much to give ophthalmologists an insight into the basic problems of their specialty, and should greatly stimulate clear thinking. The book is likely to establish itself as a standard volume.

A. Sorsby

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KIENBÖCK'S DISEASE

Jacques Rudolf Rüttner: *Beiträge zur Klinik und pathologischen Anatomie der Kienböckschen Krankheit (Lunatummalacie).* Benno Schwabe, Basel, 1946. 4 francs.

Since Kienböck first described an unusual disease of the os lunatum characterized by softening and sclerosis, deformity, axial compression and even fragmentation, much discussion has followed about its nature. Roughly speaking, three main views have been advanced. It has been suggested that there is initial trauma, with secondary dystrophic changes in the bone leading to deformity, and certainly the frequent history of a primary fracture has given support to the view. A second theory holds that a disease of the bone is concerned, and that the other features result from this. Axhausen, for instance, has emphasized aseptic necrosis of the os lunatum due to embolism, micro-organisms of low virulence lodging in the vessels of the bone and producing a bland infarct. Others have described endarteritis obliterans or vascular spasm as the mechanism of infarction. The third view would place Kienböck's disease with the more general osteopathies such as malformations, metabolic upsets, deficiencies in vitamin and hormone supply.

In a recent study of 14 cases, Rüttner gives good reasons for regarding the pathological picture from a dynamic point of view, for the variable features depend upon intensity and duration of mechanical influences and how long the reactive process has been going on. The dominating histological change is a disturbance of healing of a fracture. Primary callus is formed, but undergoes degeneration and softening, leading to the formation of secondary fractures and clefts. These may be complete or partial, so that only small areas of the os lunatum are affected; on the other hand, deformity may be pronounced. Pseudarthroses often form, and disturbances of cartilage and spongy bone are not uncommon. The latter include aseptic necrosis, cyst formation, medullary fibrosis, lysis and sclerosis of the true bony substance. Rüttner found no sign of primary necrosis in his cases, all of which gave clinical and roentgenological evidence of traumatic fracture in the first instance. He suggests that displacement of a fragment through the subchondral cortical ring of bone results in weakening of the spongy tissue because of lacunar resorption and softening immediately around the site of fracture, and predisposes to further fractures at a later date. In such a way are laid the foundations of a progressive pathological process, and from this comes the pseudarthrosis, atrophy of the spongy tissue with splitting, cyst formation and the rest.

With so many processes at work in the components of the os lunatum it is not surprising that a highly complicated *x*-ray picture is seen in the fully developed case, but a rational explanation follows from the pathological studies outlined above.

Treatment of Kienböck's disease is still far from satisfactory and Rüttner concludes that conservative therapy is better than extirpation of the bone. A follow-up of a number of his cases showed that functional disability often persisted in the joints concerned when operation was performed.

This is an excellent little monograph, full of useful information and written with a pleasing economy of words. Of the fifty odd references listed, one only is in English. Presumably a Swiss investigator can at least read that language.

G. R. Cameron

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INFECTIOUS ANAEMIA

G. Hemmeler: *L'anémie infectieuse.* Benno Schwabe, Bâle, 1946. 5 francs.

Dr. G. Hemmeler has written a monograph of 76 pages on the anaemia associated with infection. He presents in some detail his own studies of the peripheral-blood and sternal-marrow pictures in a large series of cases of different types of infectious diseases, and also discusses some experimental observations on rabbits. He concludes that the anaemia is due to a toxic action on the marrow, causing hypoplasia of erythropoietic elements.

Unfortunately no post-mortem reports on the character and extent of the marrow are given. He concludes from the sternal puncture alone that there is a hypoplasia of erythropoietic elements. It is generally recognized as important to know exactly how much active marrow there is in the skeleton before attributing any form of anaemia to marrow insufficiency.

Dr. Hemmeler confines his review of the literature almost entirely to continental work, and he appears unaware of important observations that have been published in England and America in this field.

His own observations offer nothing new in the way of facts, and his theories appear to be based on insufficient evidence.

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FRENCH CLINICAL EXPERIENCE OF PENICILLIN

R. Martin, F. Nitti, B. Sureaud & J. Berrod: *La pénicilline et ses applications cliniques.* Editions Médicales Flammarion, Paris, 1945.

The authors of this book are all members of the staff of the Institut Pasteur and its hospital, and have been working on penicillin, at first with very scanty material and information at their disposal, since late in 1943. While, therefore, a large part of the book is inevitably based on the work of foreign writers, the authors have been able to make a considerable contribution from their own experience.

The book is divided into two parts: the first deals with the biological properties of penicillin and its preparation, while the second—by far the larger section—is purely clinical. This section is subdivided according to the type of infection to be treated. It contains chapters on: Infections due to staphylococci, streptococci and pneumococci; meningitis; pulmonary infections; venereal diseases; war wounds; oto-rhino-

laryngology ; ophthalmology. (The chapter on ophthalmology is contributed by Dubois-Poulsen, while Marc Iselin and André Aubin collaborated in those on pulmonary infections and oto-rhino-laryngology respectively.) The illustrations are numerous and of a high standard and there is a bibliography of 662 references.

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GEOMEDICINE

Richard Upjohn Light : *The progress of medical geography.*

J. K. Wright : *A proposed atlas of diseases, with a note on the terminology of certain map symbols.*

Reprinted from Geogr. Rev., 1944, **34**, No. 4, pp. 636-654. American Geographical Society, New York.

Garrison, the American medical historian, pointed out in 1932 that a new science of geomedicine was in process of being born, and Sigerist, director of the Institute of the History of Medicine at the Johns Hopkins University, has favoured the publication of an atlas showing the geographical distribution of diseases in time and space. In 1944, the American Geographical Society sponsored the project for an atlas of diseases, to be designed primarily as a tool for research rather than as a popular text-book.

For the proposed atlas there are two methods of approach according to circumstance : the first is to attempt to narrow the selection down to the two or three best average projections ; the second is to treat each disease as a separate problem and to use the projection best suited for showing that particular disease. A comprehensive plate illustrates the range of symbols for use on maps ; qualitative symbols for differences in kind only and quantitative symbols for differences in degree as well as in kind. The inadvisability of trying to crowd too many kinds of data on one map is wisely emphasized.

[The pilot project for the proposed Atlas of Diseases is reviewed below.]

Anastasia van Burkalow : *Fluorine in United States water supplies.* Reprinted from Geogr. Rev., 1946, **36**, No. 2, pp. 177-193. American Geographical Society, New York.

A small amount of fluorine, about 1 part per million, is necessary for optimal dental health. Where the fluorine-content of the drinking water is much less than this, the dental-caries experience rates are high ; where it is greater, the disfigurement known as dental fluorosis, or mottled enamel, is endemic. Because of this direct relationship between dental health and an element of the physical environment, the water supply, a study of the problem was chosen as the pilot project for the American Geographical Society's proposed Atlas of Diseases, the primary purpose of which is to show the correlation of disease with the natural and social environment.

The inclusion of a fluorine test is of recent development in water analysis, and incompleteness of data is therefore one of the major problems to be faced. Suggestions are made as to how present knowledge can best be utilized for epidemiological study in the United States.

Information on the amount and distribution of fluorine in rocks is still far from complete, but enough is known to indicate that it is present in markedly differing amount in different kinds of rocks. Fluorine in soluble forms may be expected in large amounts where two conditions prevail : (i) an abundance of fluorine-containing minerals, supplied either directly from magmatic sources, or indirectly by the weathering and erosion of igneous rocks ; (ii) an abundance of pyrite, usually found in association with concentrations of organic material, to facilitate the decomposition of fluorine minerals. The need for better data on the fluorine content of potable and agricultural waters is urged upon county and state health authorities. Tests should be repeated on the same wells at different seasons. The results should be considered in relation to the depth of wells, to populations using the supply (both human and animal) and, wherever possible, with rock analysis at well depths.

Investigations on the lines suggested might well be considered together with the published findings of research on endemic fluorosis already carried out in Britain. Comparative surveys in different countries would rapidly increase our knowledge of fluorine and its various manifestations. The present communication is therefore to be welcomed. It may be pointed out that while the human bone lesions in endemic fluorotic areas of India, Argentina and South Africa are mentioned, those described in Texas in 1943 by Linsman and McMurray are not considered.

941

MEDICINE AND SURGERY IN THE USSR

V. P. Filatov : *[Optic transplantation of the cornea and tissue therapy.]* [in Russian]

Professor Filatov's fundamental work on the transplantation of the cornea and the subsequent tissue therapy has opened a new era in ophthalmology. This book is based on his experience of over 1,000 transplantations. It is a most detailed account with excellent illustrations and diagrams. The first part deals with the transplantations proper and the second with the therapeutic use of conserved tissues in the diseases of the eye, as well as in diseases which are not connected with the eye. It should be of invaluable interest to all ophthalmologists.

U. M. Oofliand : *[Modern methods of electro-diagnosis in gun-shot wounds of the nervous system.]* [in Russian]

Diagnosis by means of electricity of injuries of the nervous system was invaluable during the war. It not only confirms the diagnosis, but is also most useful for the prognosis and treatment, especially as regards the necessity of surgical interference. It is mostly used for injuries of the peripheral nervous system. This little book gives a compact but detailed account of all its uses. The author considers that the chronaximetrical method is the most perfect method of electro-diagnosis.

A. N. Obrosov : *[The technique of physiotherapy.]* [in Russian]

This short manual on the technique of physiotherapy is meant for doctors and sisters of physiotherapeutic departments of hospitals. It gives details of the construction of physiotherapeutic apparatus, stationary and portable. It also deals with the electrical processes, which transform the electric current into different therapeutic agents. There is a chapter which describes the possible defects of the apparatus and how they can be avoided by the workers using it. It is a short but very useful manual for the personnel of the physiotherapeutic departments of hospitals.

Yudin : *[The surgical treatment of gastric and duodenal ulcer.]* [in Russian]

This is based on Professor Yudin's 25 years' experience in that sphere of surgery. From 1928-1943 Professor Yudin performed 5,322 operations for various conditions which are tabulated under (*a*) malignant growths, (*b*) chronic ulcers, (*c*) perforations, (*d*) haemorrhages, and (*e*) other causes. It is a short book, but it gives a very concise account of all these conditions and the kind of operation performed. It contains a number of excellent photographs of the various stages during the operations. The final chapter deals with the gastric and duodenal ulcers in war-time. This is a most interesting and valuable book by an acknowledged master of surgery.

V. N. Sheiniss : *[The treatment of injuries of fingers and the wrist during the war.]* [in Russian]

This little pamphlet deals with the first aid, the débridement, the immobilization and the subsequent treatment of injuries of the fingers and the wrist.

[The organization of scientific work in institutions of public health in the U.S.S.R.] [in Russian]

This is a report of a meeting of the Scientific Medical Council, which was held in Moscow on April 18th-20th, 1945. It contains a short report of 9 eminent professors of the USSR dealing with their respective subjects, followed by an address by the Minister of Health, A. F. Tretiakova. It gives also an account of the discussion and ends with an order by the Minister of Health on the activities of the scientific medical organizations and a resolution of the Council.

H. W. Swann

Book Reviews

[The prices quoted are those which obtain within the United Kingdom. Editors of overseas medical journals who wish to review publications of which notices appear are invited to apply to the Editor for review copies, of which a few are sometimes available. Orders for any of the publications mentioned may be sent to the Editor if there are difficulties in obtaining them locally. Publications may be referred to by the numbers given at the left of each item, e.g. Book **942.** *It should be noted that supplies of all publications are limited and there can be no certainty that publications ordered or requested for review will be available. Publications are classified according to the Universal Decimal Classification and the classification number of each publication is given at the right.]*

ANATOMICAL EPONYMS

942 611 (92)

Anatomical Eponyms : being a Bibliographical Dictionary of those Anatomists whose Names have become incorporated into Anatomical Nomenclature, with Definitions of the Structures to which their Names have been attached and References to the Works in which they are described.

Jessie Dobson. LONDON, BAILLIÈRE, TINDALL & COX, 1946. ix + 240 PAGES. 1 PLATE. 22 × 17 cm. £1 10s. [£1,5]

Personal names are no longer recognized in official anatomical nomenclature, but are still frequently used. Professor Wood Jones, in his foreword to Miss Dobson's biographical dictionary, suggests that the use of eponyms is a main incentive to learn the history of anatomy. Miss Dobson provides short summaries of the careers of eponymous anatomists and reference to the original descriptions of the parts or processes to which their names are attached. She points out that, while in biological nomenclature personal names are often given to species in honour of scientists not directly concerned with their discovery, in anatomy it is usually the discoverer whose name is recorded. Her references to original publications are thus most valuable in establishing authority for the use of eponyms, and will be of permanent use for reference ; unfortunately they are not as precise as could be wished.

Her records clearly represent much research, as do her corrections of wrong attributions ; for it often happens that a discovery recorded by an obscure anatomist is credited to a more famous namesake. Thus she shows that "Bergmann's cords" have been reputably attributed to Ernst von Bergmann, the surgeon, while they were, in fact, described five years before his birth by G. H. Bergmann, a little-known neurologist.

The author has ascertained the correct dates of birth and death, etc., where variant dates have been recorded, and her facts seem generally accurate. The biographical notes are of varying value and insufficiently formalized ; and little attempt has been made to indicate the relative importance of the different anatomists ; for instance, there is no indication of the greatness of a Haller or a Cajal. There is uncertainty about the use of vernacular or latinized names, which could have been surmounted by fuller cross-references. More-careful editing and more precision in detail would have made the book considerably more worthy of the great learning which has gone into its compilation, but it is all the same an indispensable reference tool.

W. R. L.

APPENDICITIS

943 616.345.2-002

Patients and Appendicitis

Sir Crisp English. LONDON, J. & A. CHURCHILL, LTD., 1946. vii + 155 PAGES ; 4 ILLUSTRATIONS. 22 × 14 cm. 10s. 6d. [£0.525]

(i) Patients ; (ii) appendicitis : general aspects ; (iii) acute appendicitis : clinical symptoms and signs ; (iv) treatment of acute appendicitis : general principles ; (v) operation for acute appendicitis ; (vi) after-treatment of acute cases ; (vii) non-acute appendicitis ; (viii) indications for operation upon the non-acute appendix ; (ix) points in technique ; (x) operation on the non-acute appendix ; (xi) modern anaesthesia ; (xii) the follow through. Bibliography. Index.

This is a little manual dealing with the many aspects of appendicitis. The author has prefaced his account by a short essay on the various types of patients with whom a surgeon has to deal ; though this chapter has little direct reference to the rest of the book it is full of practical wisdom.

The rest of the book follows the accustomed lines but is of rather unequal merit. We think the detailed account of the pre-operative preparation of the patient, the enumeration of the main points of technique, and the remarks on after-treatment call attention to many valuable measures. On the other hand, the section on diagnosis is neither full nor accurate enough. The author, whose experience must have been very great, has failed to realize the difficulties of those less experienced. Otherwise why should he say on page 39, "Diagnosis is usually obvious . . ." and, two pages later, ". . . mistake is easy". No doubt it is a misprint on page 34, which allows it to be said that the first symptom is ". . . often first felt between 2 P.M. and 5 P.M.", but we consider that it is quite as erroneous to state that the initial vomiting in appendicitis is often accompanied by hiccough, or to assert that "Nausea and vomiting are often entirely absent . . ." Moreover, readers would have been more pleased to have the author's own account of the valuable points in differential diagnosis than an enumeration taken from a current text-book, however good.

There are certain omissions in the section on treatment. The operator who is opening an appendix-abscess is advised to let the pus out gradually and control the flow by a series of swabs ; no mention is made of the invaluable help afforded by suction, which will easily keep the field dry and avoid escape of pus into the peritoneal cavity. In the description of the Ochsner treatment it is advised that nothing should be given by mouth or rectum, but no mention is made as to how the patient's fluid-balance is to be maintained. Moreover, we imagine few surgeons would agree that morphine should be given four-hourly, especially since no details are given as to the dose required.

Though we agree with the author's view that repetition is often useful, we think that there was no need to describe fully the various possible positions of the appendix in three different places (pages 24, 28, 95). To sum up—this is a readable account of appendicitis, not full enough in the section on diagnosis, but helpful to the practitioner who wishes guidance on pre- and post-operative treatment. It is doubtful if the experienced surgeon will find it of much value.

CHEMISTRY IN MEDICINE

944 577.1

An Introduction to Biochemistry

William Robert Fearon. THIRD EDITION. LONDON, WILLIAM HEINEMANN (MEDICAL BOOKS) LTD., 1946. x + 569 PAGES. 22 × 14 cm. £1 1s. [£1.05]

Part I. Elements and Inorganic Compounds. (i) The subject matter of biochemistry ; (ii) biological elements ; (iii) inorganic compounds ; (iv) solutions and colloidal systems. Part II. Organic Biochemistry. (v) Classification and characteristics of organic compounds ; (vi) carbohydrates ; (vii) reactions of carbohydrates ; (viii) proteins ; (ix) amino acids and protein structure ; (x) lipides ; (xi) steroids ; (xii) pigments : pyrrole derivatives, carotinoids, flavins, melanins, etc. ; (xiii) catalysts ; (xiv) nutrients ; (xv) alimentary digestion ; (xvi) intermediate metabolism : carbohydrates ; (xvii) intermediate metabolism : proteins ; (xviii) intermediate metabolism : lipides ; (xix) tissue respiration and energy exchange ; (xx) purines and pyrimidines ; (xxi) nitrogenous bases ; (xxii) urea ; (xxiii) excretion ; (xxiv) hormones ; (xxv) the internal environment : blood and tissue fluids ; (xxvi) tissue chemistry. Appendix I. Food composition tables. Appendix II. Reagents. Index.

Many text-books of biochemistry, particularly those launched more especially at the medical student, steer a somewhat unsteady course between the Scylla of chemistry and the Charybdis of physiology. Professor Fearon is, however, an expert helmsman, and the third edition of his book demonstrates clearly that the subject of biochemistry is very much more than an undigested mixture of chemistry and biology. The author of this text-book is peculiarly well fitted for the task which he has undertaken, since he is a chemist with an expert interest in analytical technique, as well as a medical man with a wide biological outlook.

The third edition of this book differs from the previous ones in the emphasis that is now placed upon certain aspects of the subject which are of particular interest in clinical medicine ; furthermore, the chapter on "Nutrients" has been almost entirely rewritten, and a new chapter added on "Tissue chemistry". Since the majority of those reading this book will be medical students, it is right that the author should stress subjects of especial interest in medicine ; but despite this con-

cession, Professor Fearon never falters in his wider treatment of the subject, a point that is perhaps of greater significance in the education of the medical student, as well as in that of the student of science, than the particular emphasis of matters of vocational importance in medicine. A typical example of his incisive, albeit somewhat forthright, treatment of the subject, based on a wide background, may be quoted from the section concerned with the biological significance of potassium in higher land-animals: "Potassium, maintaining a vegetable tradition, accumulates in the tissues; sodium, true to a marine ancestry, circulates in the fluids." (page 16).

This book conveys in a vivid fashion the unifying power which the physical sciences wield in relation to the most diverse biological phenomena. In the reviewer's opinion, therein lies much of the importance of biochemistry to the medical student. During his pre-clinical period he should not merely equip himself with the technical information required to walk the wards intelligently; this immediately utilitarian aspect of the importance of chemistry in relation to medicine is but one part of the whole. In his early years the medical student has an opportunity, which he never again acquires, to consider the principles which underlie biological phenomena in general. In biochemistry he can learn to regard biological systems—the cell of the protozoon and the tissues of the higher animal alike—not only as subjects for visual examination on macroscopic and microscopic planes, but also as objects for consideration on a sub-microscopic, molecular basis. In learning to regard living tissues in terms of molecules, atoms, ions, and the forces acting between them, the student of medicine creates a bond with his fellow in science which can illuminate his pathway, not only through his clinical studies, but also through the years of future medical advance, for it is sure that the advance of medicine in the future must depend more and more on the application to biology of the basic concepts of chemistry and physics.

The medical student who studies inorganic chemistry early in his course sometimes finds but little straightforward connexion between his early work and his later studies in biochemistry and physiology. In Professor Fearon's book he finds two chapters devoted to the biological significance of elements and of inorganic compounds, and a most interesting discussion of the position in the periodic table of elements of particular biological importance. In this and in many other ways the book shows clearly how chemistry underlies the general phenomena of biology.

The book is brilliant in conception and excellent in general accomplishment. Such excellence makes it the more necessary to consider some matters of detail which are not wholly acceptable. From the section on pages 393 and 394, concerning "Energy exchange in biological reactions", the reader may fail to distinguish between free energy and heat, and the terms "endergonic" (Coryell) and "endothermic" appear to be regarded as synonymous. Endothermic reactions can sometimes proceed spontaneously, although it is true that endergonic reactions, both in living tissues and in reactions in non-living systems, must be coupled with a course of free energy if they are to proceed to an appreciable extent.

Dogmatic statements are sometimes made about matters on which, even for the elementary student, it might be better to express some caution. For instance (page 328), "Contrary to what was once believed, sugars have no *ketolytic*, or ketone-destroying, effect; their action is anti-ketogenic in that they provide a more acceptable source of energy than the fats . . ." Recent evidence suggest that sugars may exert a ketolytic action in providing the units of tricarboxylic acid cycle by means of which the ketone bodies may be combusted. Contrary to what is stated baldly on page 500, the pituitary growth-hormone is not lipoid in character. "Oestrogenic factor" is a most confusing term to apply to pituitary follicle-stimulating hormone (page 501). The more recent evidence that nerve-degeneration in vitamin-A deficiency can result from mechanic injuries caused by pathological overgrowth of bone (Mellanby) might be mentioned with advantage on page 271. In the reviewer's opinion it is a pity that purine and pyrimidine rings are not throughout drawn in hexagon form, instead of in the old Germanic square fashion.

In spite of these minor faults, the book shows in its conception a wideness of approach to the subject which, combined with a vivid but scholarly style of writing, makes it the most readable of text-books; while, at the same time, it contrives to be an important book of reference on all aspects of the subject. The general principles underlying the biochemical approach to the problems of life have found their most able expositor in Professor Fearon, whose book may well be regarded as echoing and amplifying the works of Wordsworth:

> "To every Form of being is assigned",
> Thus calmly spake the venerable Sage,
> "An active Principle:—howe'en removed
> From sense and observation, it subsists
> In all things, in all natures; in the stars
> Of azure heaven, the unenduring clouds,
> In flower and tree, in every pebbly stone
> That paves the brooks, the stationary rocks,
> The moving waters, and the invisible air."
> (The Excursion—Book Ninth).

It is to be hoped that, through the medium of this important "Introduction to biochemistry", Professor Fearon's inspired attitude to the subject will exert the widest influence on the development of biochemical thought.

F. G. Young

945 616-076

Micro-Analysis in Medical Biochemistry

E. J. King. LONDON, J. & A. CHURCHILL, LTD., 1946. viii + 168 PAGES; 16 ILLUSTRATIONS. 22 × 14 cm. 10s. 6d. [£0.525]

(i) Normal values; (ii) procedures for whole blood; (iii) procedures for blood plasma; (iv) procedures for blood serum; (v) procedures for cerebro-spinal fluid; (vi) procedures for faeces; (vii) procedures for urine; (viii) analysis of calculi; (ix) gastric analysis; (x) hydrogen ion concentration; (xi) spectroscopic procedures; (xii) tests of function; (xiii) volumetric solutions; (xiv) colorimetric and photometric measurements. References. Index.

This new publication is an account of the current methods in use at the laboratories of the British Postgraduate Medical School, a large part of the material being a collected account of the methods developed and improved by the author and his co-workers. The book is concise and gives a complete account of the methods and of the preparation of the reagents required, but an omission in the latter respect is the preparation of Folin and Ciocalteau's reagent. The applications of photometric principles are adequately presented. The author has attempted to cover the field of the common examinations of blood, urine, faeces, etc., but the amount of space allotted has apparently required the omission of such topics as the detection of sulphonamides in the urine.

The emphasis throughout the book is on the practical side, and it is to be regretted that such topics as the cleaning and calibration of glassware were not included, if necessary, to the exclusion of the brief references to clinical applications.

This book should find a welcome place on the laboratory shelf of the clinical pathologist and will also be invaluable to laboratory assistants, especially those reading for the final examination for the Associateship of Laboratory Technicians.

946 54 : 61

Practical Chemistry for Medical Students

William Klyne. EDINBURGH, E. & S. LIVINGSTONE, LTD., 1946. xvi + 460 PAGES. 22 × 14 cm. £1.

Part I: Some Fundamental Scientific Ideas. (i) The nature of scientific work; (ii) general ideas on qualitative work; (iii) general ideas on quantitative work. Part II: Practical Methods. (iv) manipulation; (v) measurement. Part III: General and Physical Chemistry. (vi) principles and their applications; (vii) volumetric analysis; (viii) experiments in physical chemistry. Part IV: Inorganic Chemistry. (ix) reactions of common inorganic substances; (x) identification of simple inorganic substances. Part V: Organic Chemistry. (xi) principles; (xii) reactions of organic radicals; (xiii) classification of simple organic compounds; (xiv) compounds of biological importance. Appendices. Index.

This is a new book and an excellent one. Its scope is governed largely by the present medical curriculum in the University of Edinburgh, where general and inorganic chemistry, together with some physical chemistry, are studied, either in a pre-registration course within the University or in an equivalent course at school, before the student is admitted to the faculty of medicine. More physical chemistry, together with organic chemistry, are then studied during the first year of the medical curriculum. In this respect the practice in the University of Edinburgh is similar to that in the majority of English medical schools. A difference exists, however, in that in Edinburgh the course in biochemistry during the second year of the medical course is taken in the department of physiology, despite the existence of an active department of biochemistry in the University. While the closest co-operation is desirable between the departments of physiology and of biochemistry (and indeed between the department of

anatomy and the other pre-clinical departments) in the teaching of medical students, the fact that there is a possibility of little or no co-ordination between the teaching of the introductory inorganic, physical, and organic chemistry, on the one hand, and biochemistry proper on the other, is not one that will commend itself to all unreservedly.

The scope of the book is indicated by the headings of the main sections (see above).

The 23 pages devoted to fundamental ideas are most valuable, and their influence runs like a streak of silver throughout the book. Too often the student of medicine studies elementary chemistry in bewilderment, missing the relationship between what he is doing in the laboratory and what he has heard in his lectures and failing to appreciate the nature of scientific thought and evidence. As Dr. Klyne points out, frequent reference to the earlier chapters on scientific method will be necessary if the student is to get the most out of his laboratory work, and if he is to realize that such ideas are not confined to chemistry, but are common to all organized thought. Although it cannot be denied that the early teaching of the medical student should include tuition in the nature of evidence and the organization of thought, it is perhaps surprising that Dr. Klyne's early chapters are, in a sense, an innovation in an elementary text-book of chemistry.

The section on general and physical chemistry is excellent, and the study of such general topics before the systematic study of inorganic and organic chemistry is undertaken is of great importance. There is one possible disadvantage in learning about such matters somewhat early in the course which ought to be guarded against, and that is a failure on the part of the student to recognize the general nature of the phenomena which are initially being studied in particular relationship to chemistry.

On pages 92-93 the statement is made, "Figures such as 10^{-7} or 0.0000001 are clumsy to use; hydrogen-ion concentrations are therefore usually described by means of the *negative logarithm of the concentration*". The word "therefore" in this sentence is perhaps not the happiest of choices. It is true that figures such as 10^{-7} are clumsy in use, but surely the reason for the use of the logarithmic function is not merely convenience of notation, but because the influence on biological systems of a change in hydrogen-ion concentration is determined by the ratio of the final to the initial hydrogen-ion concentration rather than by the magnitude of the increment itself. At the stage at which he first appreciates this fact, the medical student should be led to realize that a logarithmic function is of fundamental importance in biology. Even with the elementary student it could be indicated that much of his knowledge of the external world is transmitted to him by way of logarithmic functions (e.g. sight, hearing and sensory perception generally), and that the significance of logarithms in biology is certainly not limited to their use in the *p*H scale.

In his treatment of *p*K values Dr. Klyne shows himself refreshingly aware of the difficulties encountered by the elementary student in this aspect of the subject. The student who meets *p*K values only with respect to the Henderson-Hasselbalch equation has little opportunity of appreciating the general significance of *p*K's, and in many instances never grasps the distinction between "dissociation constant" and "degree of dissociation". But by the use of such approximative equations as $pH = \frac{1}{2}pK^a - \frac{1}{2}\log.\ [\text{acid}]$ and $pH = 14 - \frac{1}{2}pK^b + \frac{1}{2}\log.\ [\text{base}]$ (both to be found in this book together with other related equations) the student himself is able to check, by means of simple measurements, the relationship between *p*H, *p*K and molecular concentration in solutions of weak monobasic acids and monoacidic bases of moderate concentration.

In the section on inorganic chemistry, the reactions of common inorganic substances are related to the periodic table in an orderly fashion, and this treatment lies at the roots of the chapter on the identification of anions and of cations (group separation). This is a first-class section, and the author is to be congratulated for the successful packing of so much useful information into a logical sequence within the scope of only 121 pages. The student who masters this section cannot possibly obtain the impression, not uncommon among elementary students even a few years ago, that the group separations employed in the identification of cations were created by a separate action of inspiration which divorced them from any other aspect of chemistry.

The section devoted to organic chemistry provides an excellent introduction to this aspect of the medical student's studies, and satisfactorily relates it to what has gone before. This section as a whole perhaps lacks some of the inspiration of the other parts of the book, but it nevertheless provides an introduction to the study of more advanced biochemistry which should be most valuable. The reactions and properties of substances of biological importance are emphasized wherever their consideration is appropriate, but the mistake is not made of assuming that such matters should be introduced at the expense of the general development of the subject in its theoretical and practical aspects.

Throughout the book admirable discipline is introduced into the methods of approach to the problem in hand, the analysis of the findings and the recording of the results. The student who follows the advice of this book cannot fail to be impressed by the immense value of orderly technique.

Although Dr. Klyne's book is a practical text, its emphasis of the theory underlying practice is admirable and apposite. Modern views on the nature of valency and the structure of matter are judiciously related to laboratory procedures, and with this book the student can go from theory to practice, and back again from the bench to theory, with the greatest facility. In the opinion of the reviewer this is a point of the greatest importance, and should be a subject for vigorous development in the future. Dr. Klyne has made an excellent start in this direction and it is to be hoped that he will pursue this line with greater and greater force in future editions of this book.

It was Isaac Newton's inspiration that led him to write:

"I am induced by many reasons to suspect that they [the phaenomena of nature] may all depend upon certain forces by which the particles of bodies, by causes hitherto unknown, are either mutually impelled towards each other, and cohere in regular figures, or are repelled and recede from each other" (Preface to *Principia*).

Newton himself was unable to go far in the application of these ideas to chemical matters (see, however, Query 31 of Optics) and the application, to elementary ideas, of the results of modern investigations into the structure of liquids and solids, the nature of the forces binding molecules and atoms together, and of the conditions which facilitate the breaking and rearrangement of valency links, is but now beginning. It is to be hoped that within the next ten years the elementary books will all become orientated along such lines of development. Dr. Klyne, with the advantage of initiating a text rather than revising an old one, has already made an exceptionally good beginning. We hope that his book will exert a widespread influence on the development of the teaching of elementary chemistry, and that he himself will be carried along by the surge he will have helped to create to cover new and hitherto unexplored ground in the new editions which, in the future, may confidently be expected.

F. G. Young

947 611-018 : 541

Physical Chemistry of Cells and Tissues

Rudolf Höber, with the collaboration of *David I. Hitchcock, J. B. Bateman, David R. Goddard & Wallace O. Fenn*. LONDON, J. & A. CHURCHILL, LTD., 1945. xiii + 676 PAGES. 24 × 16 cm. £2 2s. [£2.1]

Section I: Selected principles of physical chemistry. (i) Diffusion in liquids; (ii) reaction velocity and enzyme reaction; (iii) elements of thermodynamics; (iv) electromotive force; (v) some properties of aqueous solutions. Section II: Large molecules; their physico-chemical properties and their architectural and functional significance in living matter; (vi) interatomic and intermolecular forces; (vii) some properties of large molecules in solution; (viii) condensed systems of large molecules, with special reference to the structure of fibers; (ix) some properties of films and membranes. Section III: Introductory remarks concerning the architecture of protoplasm. Section IV: The surface of the protoplast, its properties and its architecture. (x) the permeability of the cells to organic nonelectrolytes; (xi) permeability to electrolytes; (xii) permeability to weak bases and weak acids; (xiii) permeability to dyestuffs; (xiv) permeability to water; (xv) chemistry and physics of the plasma membrane. Section V: Influence of some extracellular factors on cellular activity. (xvi) the influence of inorganic ions on cell activity; (xvii) the influence of inorganic ions on fiber and cell potentials; (xviii) physiological significance of the electrokinetic or ζ-potentials; (xix) the oil-like membranes as the seat of thermodynamic potentials; (xx) the influence of nonpolar-polar organic ions; (xxi) the influence of ions upon cell potentials in plants; (xxii) the polarization of model membranes and of natural membranes by electric current; (xxiii) the influence of narcotics on cell activity. Section VI: The respiration of cells and tissues. (xxiv) The nature of respiration: (xxv) energetics and kinetics; (xxvi) the nature of oxidation; (xxvii) respiratory enzymes; (xxviii) the origin of carbon dioxide; (xxix) the relation between fermentation and respiration; (xxx) the utilization of liberated energy; (xxxi) summary. Section VII: Contractility. (xxxii) General survey of contractile tissues; (xxxiii) muscles. Section VIII: Passive penetration and active transfer in animal and plant tissues. (xxxiv) Intestinal absorption; (xxxv) the formation of urine; (xxxvi) the permeability of the body surface of animals and plants; (xxxvii) the elaboration of digestive juices; (xxxviii) some remarks about the energetics of the active transfer, the transferring device, and their mechanics.

It is a pleasure to read a scientific book in which the very praiseworthy aims of the authors are so admirably carried through as in this volume. The authors' aim was not to provide a text-book for the student of cellular physiology, but to present the achievements up to date, to show the trend of investigations, and to stimulate by pointing out the problems still unsolved.

For the full understanding and appreciation of the contents, considerable knowledge of physical chemistry and allied sciences is required; the authors, being aware of this, offer guidance to readers not fully conversant with physical chemistry by reference to certain standard text-books.

Starting with the presentation of some basic principles of physical chemistry, the authors proceed to problems of increasing complexity. Through the discussion of forces involved in the linkages of atoms and molecules, and of the properties and behaviour of molecules of high order or aggregation, we reach some of the most important sections in this book, those on the surveying of cellular structures, such as fibres, films and membranes. Much emphasis is laid on the properties of plasma membrane, and the authors, while presenting the various proposed models of this membrane as a mixed film, show that such a conception is inadequate. They propose to consider this membrane rather as an "active organ", through which solutes and solvent are often transferred by the performance of work against a concentration gradient between the inside and outside of the cell. It is implied that the work done by this cellular structure during such transfer is derived from the expenditure of metabolic energy. A parallel is drawn in this respect between plasma membrane and myosin fibrils, in which liberated metabolic energy is likewise utilized to perform work.

These considerations lead naturally to the chapters on cellular respiration, energetics and kinetics. It is shown how the potential energy of foodstuffs is liberated stepwise, and suggestions are offered how this energy might be utilized for cellular work.

The final sections on contractile tissues, intestinal absorption, renal secretion, permeability of the body-surface, elaboration of digestive juices are treated, broadly speaking, in the light of the previous chapters, and suggestions are offered for the interpretation of the mechanisms of active transfer at the various absorbing and secreting surfaces.

The book as a whole is of considerable importance; its authors have brought together a most impressive wealth of data and succeeded, as far as present-day knowledge permits, in giving a complete picture of cellular physiology. Apart from this, the work is a challenge to research workers for further investigations. Although the various sections were written by different authors, there is no feeling of discontinuity in passing from chapter to chapter, but one leaves the book with the conviction that it has been built on a solid framework. Physiologists, physical chemists, and biochemists will undoubtedly give a warm welcome to this work.

G. P.

CHILD LIFE

948 613.95 + 613.96

Child and Adolescent Life in Health and Disease: A Study in Social Paediatrics

W. S. Craig. EDINBURGH, E. & S. LIVINGSTONE, LTD., 1946. xvi + 667 PAGES; 203 ILLUSTRATIONS. 22 × 14 cm. £1 5s. [£1.25]

Part I: Historical. (i) Private philantrophy [*sic*] and voluntary effort; (ii) voluntary hospitals and the beginnings of paediatrics; (iii) poor law care of the destitute child; (iv) welfare of school children; (v) infant and child welfare; (vi) recognition of some special needs; (vii) the adolescent. Part II: Care of child life at the present time. (viii) homelessness; (ix) the juvenile in need of care and protection; (x) the maintenance of health; (xi) help for the handicapped; (xii) treatment of the sick; (xiii) preparation in child and adolescent care; (xiv) the administrative background to certain services concerned with the welfare of children and adolescents; (xv) the child under conditions of total war. Part III: The future. (xvi) The spirit of future endeavour. Part IV: Legislation relating to child and adolescent welfare. (xvii) Powers conferred on central and local authorities; (xviii) protection of the mother, foetal life and child life; (xix) concerning some aspects of child care under the poor law; (xx) concerning educational welfare; (xxi) concerning services for the medical care of certain conditions occurring in childhood; (xxii) concerning the employment of children and young persons. Appendices: I. Certain representative hospitals providing general medical and surgical care for children. II. Certain representative special hospitals, long-stay hospitals, hospital schools, residential special schools and convalescent homes for children. III. Certain representative institutions, societies, etc., directly or indirectly interested in the care of child and adolescent life in health and/or disease. IV. Categories of handicapped pupils requiring special education. V. The growth of social paediatrics: a chronological list of some outstanding events and periods. Bibliography. Index.

The title of Dr. Craig's book, *Child and adolescent life in health and disease*, is very wide, but the text does really fill in the outlines of the great subject defined by the title. In the preface he gives a concise description of the scope and aim of the book. "The present-day picture of the provisions for the care of child life and health can be compared to an unfinished jig-saw puzzle. Each piece of the puzzle has its own historical background . . . It is the aim of this book to describe the puzzle in its present unfinished form, and as an aid to completion of the puzzle to explain how past progress has been achieved."

The subject of the child and the adolescent is under active discussion at the moment, and the discussion is a political one in that there is a keen awareness that the polity of child and adolescent life is in disorder, and that its policy badly needs attention and repair. The subject is of national importance, but it is one of great complexity; and it is handled by many kinds of personnel and by many different organizations and services. The first requirement in the discussion of policy is a reliable factual account of all the agencies that attempt to meet the needs of child and adolescent life; and it is the outstanding value of the book that it supplies expert detailed information on the vast and intricate machinery, both social and medical, that ministers to all the human needs in health and in sickness of the child and adolescent. The author has had first-hand experience of administration, preceded by much clinical experience, and his book is an authoritative contribution to the problem and policy of administration in the future.

Dr. Craig is not content with describing the present and forecasting the future. He goes back into the history of the subject, and describes from the days of Queen Elizabeth the rise and gradual development of the many philanthropic and state agencies dealing with children. This first and historical part of the book is written with picturesque charm and accompanied by many quaint and telling illustrations: it is interesting in itself, but it is also valuable in explaining the position and form of the many pieces in the present-day puzzle-picture of administration.

It would take too long to describe in more detail the different parts of this remarkable book. The sub-title describes it as a study in social paediatrics. But the word *life* in the main title is the governing word. Life in the child and adolescent has its social as well as its medical aspects, and in the book the social needs of the child in education and industry are fully described. The book is, in fact, a history, a directory, and a concordance of child and adolescent life in Britain. In writing it the author has done a great service to all who are concerned with the child and the adolescent, whether they be politicians, administrators, doctors, nurses or, last and not least, mothers. Since the publication of the book, he has been appointed to the chair of child health in the University of Leeds, and he will now have the opportunity of exercising in a great city the offices of politician, administrator, physician and teacher in the field of child and adolescent life.

A final word of praise must be given to the publishers for the number and excellent quality of the illustrations.

HAEMATOLOGY

949 616.15

Disorders of the Blood. Diagnosis, Pathology, Treatment and Technique

L. E. H. Whitby & C. J. C. Britton. FIFTH EDITION. LONDON: J. & A. CHURCHILL, LTD., 1946. 665 PAGES; 15 PLATES; 71 TEXT FIGURES. 25 × 16 cm. £1 10s. [£1.5]

(i) The origin, development, functions and fate of the cells of the blood; (ii) abnormal haemopoiesis and abnormal cells found in the circulation; (iii) the principles and practice of haematological diagnosis. I. Red cells; (iv) the principles and practice of haematological diagnosis. II. Leucocytes and blood platelets; (v) the principles and practice of haematological diagnosis. III. Physical and chemical properties of the blood cells and plasma; (vi) the causes of anaemia; (vii) the nature and mode of action of haemopoietic substances; (viii) idiopathic hypochromic anaemia. The Plummer-Vinson syndrome. Chlorosis; (ix) pernicious anaemia and tropical nutritional anaemia; (x) anaemias due to disease of the alimentary tract and its associated organs; (xi) miscellaneous dyshaemopoietic anaemias. Radium and X rays. Scurvy. Thyroid disease; (xii) anaemia in pregnancy and the puerperium; (xiii) the haemolytic anaemias; (xiv) the puerpuric and haemorrhagic diseases; (xv) anaemias in infancy and childhood; (xvi) diseases due to aplasia or hypoplasia of the bone marrow. Aplastic anaemia. Agranulocytic angina; (xvii) polycythaemia, erythraemia and erythrocytosis; (xviii) the leukaemias (leucoses); (xix) miscellaneous disorders associated with splenomegaly. Splenic anaemia, Hodgkin's disease. Diseases of lipoid metabolism; (xx) infection and infectious diseases; (xxi) haemagglutination and blood transfusion. Blood

groups; (xxii) miscellaneous conditions. Allergy. Nephritis. Coronary thrombosis. Diabetes. Cancer; (xxiii) disorders involving the blood pigments. Enterogenous cyanosis; (xxiv) technique. Subject index. Index of authors.

A glance through the medical literature of the past few years is sufficient to make one realize that disorders of the blood have provided one of the most popular fields for clinical investigation. It is in order to keep abreast with this considerable output that the authors have produced a fifth edition of their well-known book only four years after the fourth. While this edition conforms to the general plan of the previous ones, the recent advances have necessitated considerable revision of the chapters dealing with haemolytic anaemias, haemagglutination and blood transfusion, the anaemias of infancy and childhood, and technique. The other chapters have been brought up to date also, the whole process involving an increase of sixty-two pages of text.

The authors' plea that cell nomenclature should be standardized will find considerable support all the world over, and the relatively simple terminology used by them could well form the basis for agreement. One can criticize only their faulty alinement of the haemocytoblast with the more primitive cell called haemohistioblast by Ferrata. Coloured reproductions of blood cells, which are rapidly improving as the years go by, will greatly assist in this standardization, and the four new illustrations in this book, together with the adjoining explanatory sketches, show a good way of reaching this end.

One must admit, however, that the technical reproduction of the picture the eye sees is still far from perfect, and it is to be hoped that improvement in this direction in the future will coincide with the insertion of a few more illustrations into the text which could be of real value to the reader. Important examples are the various cells of glandular fever, the different pictures obtained in marrow-smears from cases of myeloma, and the intra-corpuscular development stages of the four malarial parasites.

This book's chief value lies in its excellence as one for reference alike for physician, pathologist, practitioner and student. Much of the text could hardly be bettered, but, in the reviewer's opinion, the merit of the book would be considerably enhanced by a slight rearrangement of the subject-matter of its clinical section. It is admittedly difficult to know what and what not to include under the heading " Disorders of the Blood ". Pathological changes in blood and marrow are divisible into two main groups: one in which they form the essential lesion of a disease, e.g. pernicious anaemia and the leukaemias, and the other in which the tissues are but one of the many possible affected by the disease process, e.g. carcinoma and Hodgkin's disease. The first group is rightly discussed very fully, and forms a most important section of the book. It is suggested that if all the diseases of the second group were handled in the manner of the good chapter on the infectious diseases, the book as a standard of reference would be much improved. At the moment too much space is taken up in the description (sometimes inaccurate, as in the case of porphyrinuria) of the non-haematological aspects of some of the diseases in the group, whereas the haematological details of these and other diseases are in contrast rather sketchy. By way of examples, the possible blood-changes and the appearance of the pathological cells in marrow-smears are all that need be described in the case of the lipodystrophies; it would be much more helpful to anyone seeking information about the blood-changes which occur in periarteritis nodosa to find it under the heading of this disease, rather than under a section devoted to the rarer eosinophilic leukaemia.

The technical section at the end is a welcome feature of the book and would be improved still further by the transference to it of the descriptions of technical methods included in the previous chapters, i.e. those of the Donath-Landsteiner reaction and the Paul Bunnell test. Quick's method for prothrombin-time estimation is sufficiently widely used to be given in detail here. There has been considerable experimental work recently on the relative merits of the different methods for estimation of haemoglobin, the references to which are included in this text. As this procedure is of fundamental importance to the clinical haematologist, a critical analysis of these methods would have been very helpful.

" Whitby & Britton " is the outstanding British work on haematology and compares favourably with many of the American publications. While there is room for improvement, there is no question that it is of very considerable help to anybody interested in this branch of medical science. Its appeal can be gauged by the publication this year of a Spanish edition, and it would not be surprising if this venture were followed by translation into other languages as well.

MEDICAL EMERGENCIES

950 616

Medical Emergencies

Charles Newman. THIRD EDITION. LONDON, J. & A. CHURCHILL, LTD., 1946. ix + 117 PAGES. 21 × 13 cm. 10s. 6d. [£0.525]

(i) Poisoning; (ii) coma (including sudden paralysis); (iii) convulsions; (iv) circulatory failure; (v) haemorrhage; (vi) asphyxia; (vii) the colics; (viii) sudden insanity; (ix) sulphonamides and penicillin; (x) miscellaneous emergencies. Index.

Medical emergencies, as opposed to surgical emergencies, may be relatively uncommon, but when they do occur, they demand immediate treatment. In addition, they are liable to occur without any warning. This combination of factors means that the practitioner often finds it difficult to deal adequately with the emergency when it arises. As an aid to him under these circumstances this book can be thoroughly recommended. All the more usual emergencies are dealt with under the headings of poisoning, coma, convulsions, circulatory failure, haemorrhage, asphyxia, the colics, sudden insanity, sulphonamides and penicillin, and miscellaneous emergencies. In preparing this new edition considerable revision has been undertaken.

Treatment, even of an emergency, can seldom be standardized and Dr. Newman forestalls criticism by his statement that he has merely given those that he feels " . . . to be the best and simplest . . . without alternatives, in order to give definite advice to those who seek it ". Even so, it is impossible entirely to withhold criticism of some of his methods. For instance, in the treatment of barbiturate poisoning all that is said in favour of treatment with picrotoxin is that " it is justifiable ", while in the treatment of diabetic (hyperglycaemic) coma, there is insufficient stress upon the necessity for the administration of adequate amounts of insulin. As is probably inevitable, the section on the sulphonamides and penicillin is out of date, but statements such as " the essential treatment of lobar pneumonia is the administration of sulphanilamide and penicillin " might well have been deleted in the final stages of proof-reading. The list of conditions dealt with is extensive, if not exhaustive, but Cheyne-Stokes respiration is a curious omission. In a book such as this an adequate index is essential, and in future editions this might be amplified; for instance, there is no reference under " coma " to diabetes or hyperglycaemia. For a book which may well have a considerable circulation outside Great Britain it is disturbing to find the officially discarded " drachm " still used, while when dosage is given in the imperial system no equivalent in the metric system is provided.

On the whole, however, this is a useful practical book which the practitioner will find most helpful in dealing with medical emergencies.

NOSOGRAPHY

951 616

Rare Diseases and some Debatable Subjects

F. Parkes Weber. LONDON, STAPLES PRESS, LTD., 1946. 174 PAGES; 18 ILLUSTRATIONS. 22 × 14 cm. 15s. [£0.75]

Dr. Parkes Weber believes " that further progress in medical knowledge will largely be due to the study of ' rare ' diseases and syndromes ". Probably many doctors share his opinion, if one judges from the number of publications of rare and " unusual " cases in the periodicals. The author's name is closely associated with several of the rare diseases, either by first important contributions towards identifying a disease (e.g. the Sturge-Kalischer or Sturge-Weber disease) or by furthering the understanding of an already established syndrome (e.g. various forms of calcinoses, etc.). In this small volume, divided into 31 chapters, cases of a variety of rare syndromes and diseases, thoughts and opinions on debated medical subjects are presented, all of which have been published at one time or another in journals. The book does not present a systematic study, but might almost be called " collected papers ". The chapters are devoted mostly to clinical details of the author's own patients, and it is regrettable that pathological findings, which might

have made for a better understanding of many cases, are available in relatively few instances. The reader will find also the description of some curiosities, e.g. how the natives of New Guinea produce "experimentally" the "boar's tusk ring money". Freudian psychology and the problem of euthanasia are admittedly debatable subjects, but one wonders a little how epigrams on fire-bombs, which were "smothered with sand" by either doctors or porters, found their way into a book on rare diseases.

G. P.

NUTRITION

952 612.392.01

The Vitamins in Medicine

Franklin Bicknell & Frederick Prescott. SECOND EDITION. LONDON, WILLIAM HEINEMANN (MEDICAL BOOKS), LTD., 1946. xii + 916 PAGES; 208 ILLUSTRATIONS. 24 × 16 cm. £2 10s. [£2.5]

(i) Vitamin A (the antixerophthalmic or anti-infective vitamin; axeroptol); (ii) the vitamin B complex; (iii) vitamin B_1 (aneurin; thiamin); (iv) riboflavin (lactoflavin); (v) nicotinic acid (niacin); (vi) vitamin C (ascorbic acid); (vii) vitamin D (the antirachitic or calcifying vitamin); (viii) vitamin E (the antisterility or antidystrophic vitamin; alpha, beta or gamma-tocopherol); (ix) the unsaturated fatty acids and minor fat-soluble vitamins; (x) vitamin K; (xi) vitamin P. Index.

The second edition of this invaluable book, published some four years after the first, is a tribute alike to the authors, to the publishers, and to the vitamins. The rapidity with which knowledge is growing has necessitated an increase in size from 650 to 900 pages, and opportunity has been taken at the same time to increase the number of figures from 120 to 208. There has been extensive revision of many chapters and tables and a new chapter on the essential unsaturated fatty acids and minor fat-soluble vitamins has been added.

The book contains far more than its title indicates. The chapter for each of the vitamins, which are dealt with in alphabetical order, begins with a brief historical account of the history of the vitamin up to its isolation, identification and synthesis. This is followed by a discussion of the chemical properties, methods of estimation and its distribution in foodstuffs. The food-tables which complete this part are each compiled from a number of original sources and represent the best information available. The next section deals in detail, and in most cases admirably, with the biochemistry and physiology of the vitamin—the section for ascorbic acid, for example, contains some 40 pages—and is followed by a discussion of human requirements and the many factors which modify them. The later part of each chapter deals with the specific deficiency disorder, and associated conditions, with laboratory tests for detecting deficiencies, with treatment, and finally with the often unduly and unjustifiably large number of conditions in which treatment with a specific vitamin has been claimed to be successful.

While wondering at the immense amount of work that has gone into the production of this book—it contains 4,500 references—one cannot pretend that all the original papers referred to have been dealt with very critically or that the authors have been uniformly successful in their presentation of material. With the sections devoted to the vitamin-deficiency disorders there can be no fault to find, and the photographs illustrating them are not only among the best we have seen, but are beautifully reproduced. On the other hand, for example, the discussion of the part played by thiamin, riboflavin and nicotinic acid in the enzyme systems of which they form essential parts is in several respects misleading. The evidence upon which adenylic acid is included as a vitamin is somewhat tenuous even for the pigeon, and there are one or two papers to which reference might have been made in the section on ascorbic acid. A personal criticism is the continued use of vitamin B_1 for thiamin and of vitamin C for ascorbic acid.

It is, however, ungenerous to look for small faults in what is undoubtedly the best and most comprehensive account of the vitamins in their relation to medicine in the English language. Anyone who has a copy of this book will find himself, like the reviewer, referring to it more and more. He will also find—like the reviewer—that the information he seeks, together with references to several original papers bearing upon the point, will be easy to find in Bicknell & Prescott.

RADIOTHERAPY OF CANCER

953 616-006.46

The X-Ray Treatment of Accessible Cancer

D. Waldron Smithers. LONDON, EDWARD ARNOLD & CO., 1946. 147 PAGES; 102 ILLUSTRATIONS. 27 × 19 cm. £2.

(i) General principles of treatment of accessible cancer; (ii) apparatus; (iii) classification of accessible cancer, staging, records and assessment of results; (iv) dosage and the biological response of tumours to irradiation; (v) cancer of the skin; (vi) cancer of the lip, nose, ear, eyelids and cornea; (vii) cancer of the external genitalia; (viii) cancer of the mouth; (ix) cancer of the cervix uteri; (x) the *x*-ray treatment of cancer made accessible by surgical exposure; (xi) the treatment of lymphnode metastases. Appendix. Bibliography. Index.

Radiotherapy is still regarded by many in medical practice as the "enfant terrible" of modern medicine, and not without some justification. In the control of unsuitable guardians this relatively young branch of medicine has made, and continues to make, serious mistakes. In the hands of the trained radiotherapist, however, with a growing insight into the rationale of biological action, the infant is being tamed and can be depended upon to do his work, and not to commit the apparently capricious indiscretions which were frequent in his early days. These trends are emphasized in Dr. Smithers' book, which is an attempt to show the success which can be achieved using modern apparatus, up-to-date physical knowledge, and recently developed methods of cytological investigation in association with perfected and ingenious technical methods.

The beautiful illustrations show clearly the type of case treated, and the descriptions of physical factors involved are clear and concise, but lacking in one important particular, viz. the number of treatments given. This is an omission which should be rectified in view of the importance likely to attach to this factor in fractional *x*-ray treatment. (See Gray, L. H. (1944), *Brit. J. Radiol.* **17**, 327). The description of the *x*-ray apparatus and applicators used is clear, concise and well illustrated, and the isodose curves produced by Mayneord and his colleagues are clearly shown, with interesting combinations of the fields of the short-distance *x*-ray beams. Similar combinations are not shown for other types of *x*-ray beam, although the use of the 200 kV beam in the treatment of mouth cancer from outside could be usefully illustrated in such a book. The elegant devices for ensuring precision in treatment (the lens filter, moulds for positioning, and protection devices) deserve special mention, as does the emphasis placed on suitable records and their statistical treatment. The cytological investigations referred to are of two types: the differential cell-counts by the Spear and Glucksmann school, and the chromosome-breakage investigations of Koller. Their use is not clearly emphasized, however, and one differential count (Fig. 39), which is obviously unfavourable, and yet seemed to be associated with a favourable clinical response, requires some modification of the general theory to produce the truth of the matter.

A short account of the action of radiation on malignant cells puts very clearly the salient points of recent discoveries by Lea and Catcheside, Mitchell, Casperson and Santesson, and Koller, which should be very useful to those unacquainted with this work. It is refreshing to find a statistical test of results in a book of this nature, but it would be sounder to compare the figures of fractionated- with single-treatment results of skin cancer in the same rather than in different clinics. The results would still show a significant difference, although the advantage of fractionation is not proved without trials of other doses in the single-treatment method.

The chapter on mouth cancer deals with a most difficult and important region too briefly, and the arguments against adequate prophylactic treatment of lymph nodes are not consistent with the view later expressed that "when the primary growth is being treated by external radiation, fields should be arranged to include likely sites of lymph node involvement". However, it has been said that consistency is the refuge of small minds.

The book is a monograph rather than a text-book, and the author is to be congratulated on a clearly and concisely written, well-illustrated presentation of the subject, although within a limited framework, based on his own experience, backed by sound knowledge and insight into the difficult subject of which he writes. The publishers and printers also merit commendation for the paper, type, illustrations and general format, which add to the pleasure experienced in its reading.

F. Ellis

Films

The Scientific Film Association and the Royal Society of Medicine have been co-operating in the preparation of a reference catalogue of all the films of medical interest in Great Britain. Dr. Stanford, who was in charge of this work, gives below a brief account of how it was done.

954

MAKING A CATALOGUE OF MEDICAL FILMS

BRIAN STANFORD, M.R.C.S., D.M.R., F.R.P.S.

Until now a teacher of medicine in Britain wishing to use film has been unable to do so for lack of a unified catalogue listing the films available and suitable for his purpose. A few collections have been listed by title, but the vast majority of films lie unused because their existence is unknown. The difficulty of obtaining suitable films has led many teachers to make their own, with various degrees of success; but when an insistent request has come forward to make a film from university funds, the decision to undertake production has often been impeded because of the possibility that a film on the subject might already exist. A vicious circle thus exists by which films are either not made, or not used if made, and a reference catalogue is seen to be essential to progress.

This state of affairs was so well recognized by the Medical Committee of the Scientific Film Association (SFA) that their first action after formation in 1943 was to publish an appeal in the journals for information on the location of films of medical interest. Replies were collected and filed, but real cataloguing was held up for lack of adequate funds. A few months later it became known that the Royal Society of Medicine (RSM) was contemplating founding a library of medical films; and they too needed a catalogue to provide an estimate of the work likely to be involved. It was quickly evident that co-operation was both feasible and desired, and in January 1945 a nominee of the SFA, working on SFA procedure and financed by the RSM, started work. By March 1946 most of the films known to exist in Britain had been viewed and synopsized, and the material so collected is now being prepared for publication by the Association of Special Libraries and Information Bureaux (ASLIB). This catalogue will not, however, contain any description of the cataloguing-procedure, for a separate publication describing this is under consideration, but in the meanwhile the condensed description which follows may prove useful to other countries contemplating the preparation of a similar catalogue.

Data to be Collected

A mere list of titles obtained from written replies to a circular is not satisfactory. In the first place, the title so given is, in about 40% of the cases, not the same as the title on the film, which leads to much confusion, and secondly the title alone gives no indication of the content of the film: for example "Hypertrophic pyloric stenosis" may be a record of visible gastric peristalsis in an infant, or a demonstration of the method of administering Eumydrin, or a record of an operation for the condition, and so on. Also a written synopsis of the content of the film is desirable, because the film, unlike a book, cannot be "flicked through" and an impression of its usefulness gained at a short glance.

Yet even this synopsis is not sufficient; an objective summary of what the film shows can give no indication of whether it shows it clearly or badly, whether the demonstrator's hand obscures vital sequences, whether the camera is too far away to show plainly the features it is recording, whether the facts are correct but their sequence of presentation confusing, and so on. So a written criticism of the teaching value of the film, prepared by a panel competent to pass judgment, is essential to a complete film catalogue; this is the "Appraisal". The catalogue will also list the length of the film; its gauge; whether sound or silent, colour or monochrome, copy or original; and where it can be obtained. With all this information to be obtained of necessity for each film, it is reasonable at the same time to record the authority who made the film, the photographer, and similar details (the "credits"); and it is valuable to note the methods and techniques employed in making the film.

All this information is useful to the teacher who is seeking to select the film most appropriate to his needs from the catalogue listing perhaps four or five titles which might be suitable. If he is enabled to narrow his choice to only two, he has saved much time; and the film library can reduce needless circulation of films which have by nature a limited projectable life. Cataloguing scientific films is therefore a big undertaking if performed properly, but once done the work stands for all time, and is added to as new films appear.

Staff and Equipment

It was thought essential that the viewing of each film be done by a medical man, for only he can interpret the significance of the actions portrayed, or use the correct technical terms in writing the summary. This medically-qualified viewer was employed full-time on the project. In order to make the most intensive use of his specialized knowledge, it was found necessary to give him as assistants a secretary-stenographer, a copy-typist, and a general assistant who was trained to operate the projector, to inspect, repair, pack and despatch films, and to act as messenger and filing-clerk. A 16 mm. silent projector was placed at his sole disposal, and a sound machine was available when necessary. The silent machine was fitted with variable speed control (most of the viewing, even of silent films, was done at 24 frames/sec.), and reverse- and stop-mechanisms, the latter especially being needed for noting title, technical adviser, and other credits. The sound machine was not used routinely for silent films, firstly because many films were in bad condition and would not project satisfactorily on a machine having only one line of sprockets, and secondly because the lamp was overheated if the machine was used at 16 frames/sec. for long intervals.

Viewing

Viewing was mostly done in a small room, on a screen 12×16 inches [30×40 cm.], with a low level of general room-illumination. In this way an extremely bright image was viewed from a few feet away, with enough local light to take notes while the film was running. This was essential, for a synopsis written from memory after the film has finished is not satisfactory. With a little practice it becomes easy to look down at the note-pad on one's knees and to write while the film is in progress without missing essential steps: stopping the film for each note to be written would be tedious. To these rough manuscript notes were added a

comment on the quality and value of the film, and an arbitrary grading of merit: 1—excellent, 2—useful, 3—possibly usable, and 4—useless.

This grading and comment was used later in compiling programmes for the Appraisal Panels; these were presented with one film from each group—1, 2 and 3—so procuring balanced opinions; the films in group 4 were to be left until the main work was covered. The personal opinion and grading were made for the use of the cataloguer only and were never intended for publication in any form; but the value of these comments became evident later when the work on the catalogue became known, and frequent requests for assistance in programme-compilation were received. Here the cataloguer's comments, unauthoritative though they were, were found to be of great value, a point which emphasizes the need for authoritative appraisals in film catalogues.

Compiling the Records

The manuscript notes were transferred in typescript to the back of a standard form, which was then filed alphabetically. In addition, other cross-references were compiled; the film-titles were listed in numerical order, and were listed under subject headings (for instance, a film on congenital torticollis would be entered under Obstetrics, Paediatrics, and Orthopaedics); the medical man responsible for making the film was allocated a filing card and the title was put under his name. As other films with which he was connected came for viewing, the titles were added to this card.

In addition to these obvious indexes, two others were found essential: a small card, 3 × 4 inches, on which was recorded the title of the film, and the stage of cataloguing that it had reached (i.e. existence known, but not traced; called for view; held for view; returned after viewing; and so on); these were filed in alphabetical order and under no circumstances allowed out of the filing cabinet: the other was a card 6 × 9 inches, on which was recorded the title, odd notes about the film, where we had learnt of its existence (e.g. by word-of-mouth from Dr. ——, from a review in a journal, etc.), and the dates on which it was sent for, viewed, returned to the owner, etc. This card was filed according to the stage the film had reached in our cataloguing-procedure, so that we could see at a glance how many films had been sent for, how many held for viewing, how many known of but not yet sent for, and so on. In this way a smooth flow of films was maintained; to the back of these cards were stuck any reviews found published, or references to those reviews.

Tracing and Handling the Films

The last-mentioned index card was made out as soon as we learnt of the existence of a film, whether by chance remark from a colleague, in a letter accompanying some other film, or from a published reference. The source of information, as has already been mentioned, was added. In this way news of the existence of a film was recorded instantly, and the film itself could be traced at leisure. The title was entered in pencil, for the title first heard was rarely the same as that on the film when it arrived. This true title was typed on, and if the first-mentioned title was known to be widely used it was also filed on a separate card as a cross-reference to the true title. This was often found necessary, for a film would be listed in two different catalogues with slightly different titles, and it became important to recognize whether they referred to two different films or were only variants of the same title.

The owner of the film was sent a form on which the purpose of the cataloguing was explained, and on which he was asked to give a few details about the film and to say whether he would be willing to lend it for viewing. In practice this permission was not once refused, but it was important to remember at all times that the films were private property and lent as an act of courtesy; the voluntary nature of the scheme underlay all our actions. The film was eventually sent for, and on arrival inspected, cleaned, and where necessary spliced before viewing. We aimed, wherever possible, to return the film in better condition than it arrived; this was usually not difficult, for they often came in a neglected condition. After viewing, they were rewound and returned by registered post, and a covering letter was sent separately thanking the owner for the loan.

In this way some 1,200 titles were recorded in fifteen months, and 800 films were viewed and synopsized. Appraisal was started according to the system evolved by the Scientific Film Association, but only one subject-group, 26 films on anaesthesia, was covered before the main work of cataloguing was completed, and the information so far gathered prepared for publication. Appraisal is being continued by the Scientific Film Association, who have undertaken the responsibility of keeping the catalogue up-to-date.

Films

MORE FILMS FROM THE INSTITUT PASTEUR, PARIS

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M. Pierre de Fonbrune, who showed last January, under the auspices of the British Council, some of the cinemicrographic films made by M. Comandon and himself at the Institut Pasteur in Paris, gave two further demonstrations at the British Council Film Theatre on Monday, 15 July. Four of the films were reviewed in an earlier number of the Bulletin (*BMB* **812**). Reviews of three more are given here. Applications for the loan of any of these films should be made to Cinematographic Attaché, French Embassy, London.

1 CARYOCINESE

The film is chiefly devoted to karyokinesis in the mother cells producing erythrocytes in the Triton. Abundant feeding after several months of starvation produces active growth in the haemopoietic as well as other tissues, making easier the task of catching the cells when they divide. By light compression the cells are immobilized, and chromosomes, mitochondria and the rotation of the nucleus before division are demonstrated.

The movement is speeded up, and it is of considerable interest to notice lively movement of leucocytes dancing across the field. Nuclear division in a small amoeba, acanthamoeba, is also shown.

This film probably represents the best possible results to be obtained by these methods of photography, and it conveys, as only the film can, a vivid over-all impression for the student of this fundamental event of life, and for the research biologist details of the event, otherwise difficult to record, for close study at leisure.

2 AMOEBA VERRUCOSA

The amoeba is seen engulfing long particles of alga. The alga is seized and actively pulled into the amoeba—which answers an old problem as to whether the food of the amoeba penetrated into it by passive physical means. The long piece of alga is engulfed and coiled up in the amoeba, but this coiled-up strip is under tension and sometimes it comes undone and bursts the amoeba open. The damage is, however, rapidly repaired, and the amoeba soon starts eating again.

3 PHAGOCYTOSE

This film shows speeded-up photography of blood. Attention is given chiefly to the polymorphs. Then amoeboid movements are shown, and by a lower-power view their active travelling about is demonstrated. By the use of a special micropipette, two sorts of bacteria are introduced, and the leucocytes gather round and dispose of them.

A closer analysis of the differential phagocytosis of different organisms would be possible by this method and would be of considerable interest.

The monocyte is not demonstrated, but some lymphocytes come casually into the field, and they show mobility and active contractile movements of the nucleus. These are important demonstrations, for it has been considered that the lymphocyte is immobile, and that the lobulated lymphocytes seen in lymphatic leukaemia are essentially abnormal forms.

The film would be of considerable value for teaching, and it is to be hoped that the Institut Pasteur will make it (and the other films) available to UNESCO for circulation throughout the world.

Ronald MacKeith

*

HEALTH EDUCATION FILMS

956

Your Children's Ears

made by Realist Film Unit, 1945 ; owned and produced by the Central Office of Information for the Ministry of Health, in co-operation with the Central Council for Health Education ; 16 mm. sound, 540 ft. (160 m.) ; 35 mm. sound, 1,340 ft. (400 m.) ; black and white ; 15 minutes.

This is one of a series of health films designed mainly to instil into parents the need for obtaining medical advice about certain childish ailments or infirmities at an early stage.

The film shows, by means of diagrams, the mechanism of hearing and how this can be interfered with by neglect of what appear to be comparatively trivial matters. The diagrams are clear and explain in a straightforward simple manner how the complicated ear works.

The films would not only be suitable for showing to audiences at Maternity and Child Welfare Clinics and Women's Institutes, but might with some advantage be shown to medical students when they are working on this subject.

The principal message, that one should seek medical advice for all ailments of the ear or the throat, is well emphasized and the film should be a popular addition to any health demonstration.

Amulree

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Your Children's Eyes

made by Realist Film Unit, 1945 ; owned and produced by the Central Office of Information for the Ministry of Health, in co-operation with the Central Council for Health Education ; 16 mm. sound, 690 ft. (210 m.) ; 35 mm. sound, 1,710 ft. (510 m.) ; black and white ; 19 minutes.

This film opens with a number of shots showing how children with indifferent sight are handicapped at school and in their normal life. It emphasizes the need for parents to seek medical advice for their children if they are in any doubt whether they can see properly. A number of shots are given showing the good effect of glasses on defective eyes. The film also explains, by a series of simple diagrams, the mechanics of the common squint, and conveys this information in a way which, although simple, could be usefully employed in the training of medical students and others interested in ophthalmics. The proper correction of this defect by means of appropriate lenses is explained simply by means of diagrams. The commentary is well done and the photography is, generally speaking, good.

This film should serve a valuable purpose in explaining in simple terms the structure, function and common defects of eyes and should encourage mothers to take their children promptly to hospital if they suspect their vision is not perfect.

Amulree

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Your Children's Teeth

made by Realist Film Unit, 1945 ; owned and produced by the Central Office of Information for the Ministry of Health, in co-operation with the Central Council for Health Education ; 16 mm. sound, 500 ft. (150 m.) ; 35 mm. sound, 1,260 ft. (380 m.) ; black and white ; 14 minutes.

The importance of early regular and frequent visits to the dentist is the main message of this film.

The eruption of the milk and permanent teeth is shown by means of diagrams and the process by which decay is set up is well shown by means of animated cartoons. Practical instruction on the proper way to brush the teeth is given and the film could usefully be shown to any audience of parents or children.

The method of cleaning the teeth by a rotary movement always seems to make that operation rather more difficult than it need be, and it seems a pity to exclude the more straightforward ways of handling the toothbrush.

The photography is good and the diagrams are clear.

Amulree

* * *

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The Microscope

made and owned by Realist Film Unit, 1945 ; 16 mm. sound, 410 ft. (120 m.) ; 35 mm. sound, 1,040 ft. (310 m.) ; black and white ; 11 minutes.

The compound microscope is used extensively by pupils in senior schools and universities, yet it is exceptional for them to be given clear instruction in its use when first they meet it. This film provides much of that necessary introduction. It is divided into three parts spaced by blank film so that the teacher may stop at each stage to answer questions, and teaching notes and a wall chart (which appears in the film) are provided as an integral part of the visual unit. The first part of the film demonstrates the mechanical and optical components (nosepiece, drawtube, stage, condensor, objective, eyepiece) and the lighting unit (lamphouse, bulb, iris, filter-holder). The second part shows how to set up the microscope for observation, and advocates a definite routine by which the object is first focussed while illuminated by simple transmitted light, and then the plane of the illuminator iris is focussed by the sub-stage condensor into the plane of the object. The stages of the manipulation needed are clearly shown, and the proper use of sub-stage and lamp-source irises. Part three is devoted to the adjustment of the high-power objective. Here the value of accurate focussing of the sub-stage condensor advocated in part two is reinforced, for the focussing is done on the aerial image of the illuminant instead of on the

FIG. 1. FOCUSSING THE CONDENSER

When the pencil image is in sharp focus the image of the lamphouse opening is in the plane of the slide. This gives maximum illumination.

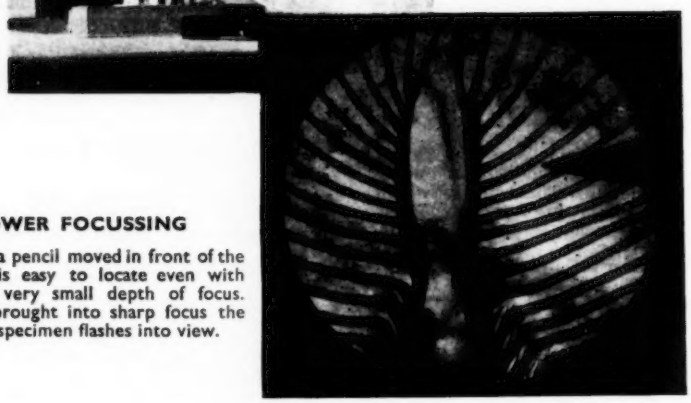

FIG. 2. HIGH POWER FOCUSSING

The image of a pencil moved in front of the light source is easy to locate even with objectives of very small depth of focus. When it is brought into sharp focus the image of the specimen flashes into view.

object ; this is easily achieved by moving a pencil-point in the plane of the illuminator iris. Once this is picked up the real object will be seen, and only minor adjustment of the fine focus is needed. In this way damage to the slide (and the objective) by accidental rapid passage through the plane of focus is avoided.

This is an essentially elementary introduction—dark-ground and oil-immersion techniques are not mentioned—which will be widely welcomed in all biology training centres, for the information is clearly and accurately presented. But it may be regretted that the construction of an objective is demonstrated by taking one to pieces, for this is a manoeuvre that should not be encouraged, and a lens shown in section would have done just as well ; while a demonstration of the amazing precision of the fine-focus adjustment mechanism could well have been included.

Brian Stanford

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It began on the Clyde

made by Greenpark Production, 1945 ; owned by the Central Office of Information for the Department of Health for Scotland ; 16 mm. sound, 540 ft. (160 m.) ; 35 mm. sound, 1,350 ft. (400 m.) ; black and white ; 15 minutes.

At the outbreak of war in 1939 the hospital services of Britain were completely reorganized and large numbers of new hospitals established outside the cities to treat the expected war casualties from both civilian and military sources ; fortunately these facilities were not immediately needed, but much skilled staff was kept idle as a result. By 1941 black-out conditions were beginning to be reflected in an increased incidence of minor disorders among factory workers, and the depleted civilian medical service was unable to devote the time required for reassuring each of these patients. Glasgow decided that as an experiment a few such patients should be admitted at their doctor's request into the almost empty Emergency Medical Service [E.M.S.] hospitals, at least until such time as direct war casualties arrived in greater numbers. This film shows how the scheme was conceived and worked, and follows the treatment of one patient admitted to an E.M.S. hospital. Here he is investigated to see whether his apparently nervous symptoms have any organic basis, he is given appropriate physiotherapy, good food and plenty of rest in country surroundings. Under this régime his symptoms subside and he returns to work. This pilot scheme worked so well that later it was extended to include surgical cases from the hospital waiting-lists in addition to these problem patients referred by local doctors. This step also proved its worth, and the Department of Health for Scotland then applied the Clyde Basin Scheme, as this pilot system had come to be called, to the whole E.M.S. of Scotland. The film ends with a plea that although this method of treating town patients in country surroundings was successfully started under the stress of war, reversion to peace-time conditions must not allow the system to lapse.

To some extent this film fails to carry conviction, partly because we cannot remember that conditions were as bad in 1941 as depicted here, and partly because the leading actor is miscast. But the film, which has been used as one of the films-of-the-month by the Ministry of Information, should be widely appreciated in these mechanistic times for the strong case it puts that comfort, attractive food, and pleasant surroundings are just as important as drugs and surgery in helping to cure a sick man.

Brian Stanford

Corrigendum

Vol 4 No. 3

BMB **876**, The genetics of cancer, by Georgiana M. Bonser. P. 207, col. 1, l. 41: " F_2 " should read " F_1 ".

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